

10/066,007

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(FILE 'HOME' ENTERED AT 15:04:15 ON 13 APR 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 15:04:54 ON 13 APR 2004

L1 3 S ASTAXANTHIN (A)SYNTHASE?
L2 19847 S "AST"
L3 5489 S ASTAXANTHIN
L4 36 S L2 AND L3
L5 1238 S RHODOZYMA
L6 6 S L4 AND L5
L7 3 DUP REM L6 (3 DUPLICATES REMOVED)
L8 212 S L2 AND SYNTHASE?
L9 0 S L5 AND L8
L10 4 S L3 AND L8
L11 1 DUP REM L10 (3 DUPLICATES REMOVED)
E HOSHINO T/AU
L12 4343 S E3
E OJIMA K/AU
L13 471 S E3
E SETOGUCHI Y/AU
L14 435 S E3
L15 5227 S L12 OR L13 OR L14
L16 5 S L2 AND L15
L17 3 DUP REM L16 (2 DUPLICATES REMOVED)
L18 66 S L2 (A)GENE
L19 28 DUP REM L18 (38 DUPLICATES REMOVED)
L20 53134 S BETA(A)CAROTENE
L21 2078 S L3 AND L20
L22 190 S L5 AND L21
L23 10081 S XANTHOPHYLL
L24 16 S L22 AND L23
L25 9 DUP REM L24 (7 DUPLICATES REMOVED)

10/066,007

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L17 3 DUP REM L16 (2 DUPLICATES REMOVED)
L18 66 S L2 (A)GENE
L19 28 DUP REM L18 (38 DUPLICATES REMOVED)

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Biotechnology technique
jul 1995 9(7) 509-512

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and searchable
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changes
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NEWS 7 MAR 03 MEDLINE file segment of TOXCENTER reloaded
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	ENTRY	SESSION
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=> s staxanthin (a)synthase?
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SEARCH ENDED BY USER

=> s astaxanthin (a)synthase?
L1 3 ASTAXANTHIN (A) SYNTHASE?

=> d 1-3 ibib ab

L1 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:281124 BIOSIS
DOCUMENT NUMBER: PREV200200281124
TITLE: **Astaxanthin synthase.**
AUTHOR(S): Hoshino, Tatsuo [Inventor, Reprint author]; Ojima, Kazuyuki
[Inventor]; Setoguchi, Yutaka [Inventor]
CORPORATE SOURCE: Kamakura, Japan
ASSIGNEE: Roche Vitamins Inc.
PATENT INFORMATION: US 6365386 April 02, 2002
SOURCE: Official Gazette of the United States Patent and Trademark
Office Patents, (Apr. 2, 2002) Vol. 1257, No. 1.
<http://www.uspto.gov/web/menu/patdata.html>. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 8 May 2002
Last Updated on STN: 25 Jun 2002

AB The present invention is directed to genetic materials useful for the
preparation of astaxanthin from beta-carotene, such as polypeptides having
astaxanthin synthase activity, DNA fragments coding for
astaxanthin synthase, recombinant organisms and the
like. Those novel genetic materials may be derived from *Phaffia*
rhodozyma. The present invention also provides a process for the
production of astaxanthin.

L1 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2000-14326 BIOTECHDS
TITLE: Novel polynucleotide encoding **astaxanthin-**

synthase useful for producing recombinant cells for producing astaxanthin from beta-carotene; plasmid pRLR913-mediated gene transfer and expression in *Phaffia rhodozyma*

AUTHOR: Hoshino T; Ojima K; Setoguchi Y
PATENT ASSIGNEE: Roche
LOCATION: Basle, Switzerland.
PATENT INFO: EP 1035206 13 Sep 2000
APPLICATION INFO: EP 2000-104430 3 Mar 2000
PRIORITY INFO: EP 20002101666 1 Feb 2000; EP 1999-104668 9 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-559874 [52]

AB A N containing nucleotide sequence coding for an enzyme with **astaxanthin-synthase** activity catalyzing the reaction from beta-carotene to astaxanthin, preferably in *Phaffia rhodozyma*, is new. Also claimed are: a vector or plasmid (e.g. plasmid pRLR913) containing the DNA; a host cell (e.g. *P. rhodozyma* (ATCC 96815)) transformed or transfected by the DNA or vector; a protein encoded by the DNA; preparation of the protein by culturing the host cell under suitable conditions and recovering the expressed protein; and production of astaxanthin by contacting beta-carotene with a peptide with **astaxanthin-synthase** activity in the presence of an electron donor in a reaction mixture containing a reconstituted membrane. The DNA and protein are useful for producing astaxanthin. (46pp)

L1 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:645717 HCAPLUS
DOCUMENT NUMBER: 133:234467
TITLE: Cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and use of the enzyme for production of astaxanthin
INVENTOR(S): Hoshino, Tatsuo; Ojima, Kazuyuki; Setoguchi, Yutaka
PATENT ASSIGNEE(S): F. Hoffmann-La Roche A.-G., Switz.
SOURCE: Eur. Pat. Appl., 46 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1035206	A1	20000913	EP 2000-104430	20000303
EP 1035206	B1	20031015		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 6365386	B1	20020402	US 2000-518386	20000303
AT 252155	E	20031115	AT 2000-104430	20000303
CN 1266101	A	20000913	CN 2000-103755	20000308
BR 2000001369	A	20010814	BR 2000-1369	20000308
JP 2000262294	A2	20000926	JP 2000-65041	20000309
US 2003077691	A1	20030424	US 2002-66007	20020201
PRIORITY APPLN. INFO.:			EP 1999-104668	A 19990309
			EP 2000-101666	A 20000201
			US 2000-518386	A3 20000303

AB The present invention is directed to genetic materials useful for the preparation of astaxanthin from β -carotene, such as polypeptides having **astaxanthin synthase** activity, DNA fragments coding for **astaxanthin synthase**, recombinant organisms and the like. Those novel genetic materials may be originated from *Phaffia rhodozyma*. Cloning, genomic and cDNA sequences of **astaxanthin synthase** of *P. rhodozyma* and amino acid sequence of the encoded enzyme are disclosed. The present invention also provides a process for

the production of astaxanthin.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s "ast"

L2 19847 "AST"

=> s astaxanthin

L3 5489 ASTAXANTHIN

=> s l2 and l3

L4 36 L2 AND L3

=> s rhodozyma

L5 1238 RHODOZYMA

=> s l4 and l5

L6 6 L4 AND L5

=> dup rem l6

PROCESSING COMPLETED FOR L6

L7 3 DUP REM L6 (3 DUPLICATES REMOVED)

=> d 1-3 ibib ab

L7 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:532320 BIOSIS

DOCUMENT NUMBER: PREV200300534283

TITLE: The carotenoid biosynthesis genes crtI, crtYB and
ast of *Phaffia rhodozyma* are located on
the same chromosome.

AUTHOR(S): Echavarri-Erasun, C. [Reprint Author]; Johnson, E. A.
[Reprint Author]

CORPORATE SOURCE: University of Wisconsin-Madison, Madison, WI, USA
SOURCE: Abstracts of the General Meeting of the American Society
for Microbiology, (2003) Vol. 103, pp. P-132.
<http://www.asmusa.org/mtgsrsrc/generalmeeting.htm>. cd-rom.
Meeting Info.: 103rd American Society for Microbiology
General Meeting. Washington, DC, USA. May 18-22, 2003.
American Society for Microbiology.
ISSN: 1060-2011 (ISSN print).

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 12 Nov 2003

Last Updated on STN: 12 Nov 2003

AB Carotenoids are naturally occurring pigments formed through the isoprenoid pathway that impart attractive color to animals and plants and also have essential biological functions as antioxidants, membrane stabilizers and as precursors to essential metabolites such as vitamin A. The food industry utilizes carotenoids as natural sources of pigmentation for enhancing the organoleptic appeal of certain foods, including salmonids and crustacea produced in aquaculture. Carotenoids have also gained attention for their potential health benefits, leading to potential new uses as nutraceuticals. The basidiomycetous yeast *Phaffia rhodozyma* (teleomorph *Xanthophyllomyces dendrorhous*) produces **astaxanthin** as its major carotenoid and is a commercial source of this important pigment. Although a detailed understanding of carotenoid biosynthesis in *P. rhodozyma* is not yet available, the structural genes crtI, crtYB and **ast** have recently been reported to code for enzymes involved in the conversion of the colorless precursor carotenoid phytoene to **astaxanthin**, and their nucleotide sequences are available. We used Pulsed Field Gel Electrophoresis (PFGE)

to analyze electrophoretic karyotypes of several wild-type *P. rhodozyma* strains. Southern blots were analyzed with ³²P-labeled DNA probes targeting the *crtI*, *crtYB* and *ast* genes. These experiments revealed that all three genes are located on the same 1.6-1.9 MB chromosome. This represents the first work describing the physical locations of genes involved in *astaxanthin* biosynthesis in *P. rhodozyma*. Further studies will focus on determining if these genes are clustered and on regulation of their expression.

L7 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 2002-11049 BIOTECHDS

TITLE: Production of carotenoids, particularly *astaxanthin*, useful as colorant in fish feeds, by culturing mutant organism with reduced alternative oxidase activity; vector plasmid pCR2.1-TOPO-mediated gene transfer and expression in *Escherichia coli*, DNA primer, antisense and polymerase chain reaction for use in fish feedstuff and as a food-additive and colorant

AUTHOR: HOSHINO T; OJIMA K; SETOGUCHI Y

PATENT ASSIGNEE: HOFFMANN LA ROCHE and CO AG F

PATENT INFO: NO 200102552 26 Nov 2001

APPLICATION INFO: NO 2000-2552 24 May 2000

PRIORITY INFO: EP 2000-111148 24 May 2000

DOCUMENT TYPE: Patent

LANGUAGE: Norwegian

OTHER SOURCE: WPI: 2002-194135 [25]

AB DERWENT ABSTRACT:

NOVELTY - Production of carotenoids (I) by growing an organism (A) produced by treating a (I)-producing organism to reduce activity of an alternative oxidase (AO) and selecting strains with increased (I) productivity.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for (1) a method for producing a mutant strain with increased (I) productivity by growing a parent organism under conditions that reduce AO activity, and selecting; (2) mutant strain produced by method (a); (3) recombinant organism with increased (I) productivity, produced by altering expression of the AO gene; and (4) recombinant DNA encoding an AO from a (I)-producing organism.

BIOTECHNOLOGY - Recombinant DNA of (d) is a 3724 bp sequence (2), encoding a 410 amino acid protein (1), from *P. rhodozyma*, or a sequence with at least 55% identity at the nucleic acid level or 51.5% identity at the protein level. Preparation: Degenerate primers (sequences given) were designed based on sequences conserved in known AO genes and used for polymerase chain reaction (PCR) amplification of cDNA from a spontaneous SHAM-resistant mutant of *P. rhodozyma*. Amplicons were cloned into pCR2.1-TOPO for expression in *Escherichia coli* TOP10. One isolated amplicon was used to screen genomic DNA from the same source to select a 6 kb *EcoRI* insert of which 2561 bp were sequenced to identify a 1206 bp reading frame containing 10 exons and 9 introns. The Genome Walker kit was used to clone the region 5' of the coding sequence to identify a 1406 bp promoter; together with the coding region this forms sequence (2). An antisense sequence corresponding to the entire structural gene of AO was amplified by PCR and cloned under control of the *AST* gene promoter and terminator. When the recombinant vector was used to transform *P. rhodozyma*, one clone with increased pigmentation was detected.

USE - The method is particularly used to prepare *astaxanthin*, used as a fish feed additive to impart an orange-red color to the flesh.

ADVANTAGE - (A) provide yields of (I) up to 50% higher than wild-type strains.

EXAMPLE - When the wild-type *Phaffia rhodozyma* was grown for 65 hr at 20 degrees C, the content of *astaxanthin* was 0.211 mg/g dry cells. When the same strain was transformed with a vector that

expressed a sequence antisense to the alternative oxidase gene, the corresponding figure was 0.240 mg/g dry cells, i.e. an increase of about 15%.

L7 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
ACCESSION NUMBER: 1995:359602 BIOSIS
DOCUMENT NUMBER: PREV199598373902
TITLE: Improvement of biochemical features in fish health by red
yeast and synthetic **astaxanthin**.
AUTHOR(S): Nakano, Toshiki [Reprint author]; Tosa, Michiyo; Takeuchi,
Masaaki
CORPORATE SOURCE: Marine Biochem. Lab., Fac. Agric., Tohoku Univ., Aoba-ku,
Sendai 981, Japan
SOURCE: Journal of Agricultural and Food Chemistry, (1995) Vol. 43,
No. 6, pp. 1570-1573.
CODEN: JAFCAU. ISSN: 0021-8561.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Aug 1995
Last Updated on STN: 30 Aug 1995

AB The biochemical characteristics of the liver and blood in rainbow trout
(*Oncorhynchus mykiss*) fed a diet supplemented by red yeast (*Phaffia*
rhodozyma) containing **astaxanthin** (**Ast**) as its
principal carotenoid pigment or synthetic **Ast** were studied.
Surprisingly, the hepatosomatic indices and serum glutamic-oxaloacetic
transaminase' activities of fish fed a diet containing red yeast or
synthetic **Ast** were significantly lower than those of fish fed a
control diet. The mean amount of serum lipid peroxide of fish fed a diet
containing red yeast or synthetic **Ast** was also lower than that
of the control fish. It was suggested that the dietary red yeast and
synthetic **Ast** have the potential for improving not only the
pigmentation of fish muscle but also the health of fish in aquaculture
(e.g. improvement of liver function and increase of defensive potential
level against oxidative stress).

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L8 212 L2 AND SYNTHASE?

=> s l5 and l8
L9 0 L5 AND L8

=> s l3 and l8
L10 4 L3 AND L8

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11 1 DUP REM L10 (3 DUPLICATES REMOVED)

=> d all

L11 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
AN 2003254848 MEDLINE
DN PubMed ID: 12766075
TI Effects of **astaxanthin** on lipopolysaccharide-induced inflammation in vitro and in vivo.
AU Ohgami Kazuhiro; Shiratori Kenji; Kotake Satoshi; Nishida Tomomi; Mizuki Nobuhisa; Yazawa Kazunaga; Ohno Shigeaki
CS Department of Ophthalmology and Visual Sciences, Hokkaido University Graduate School of Medicine, Sapporo, Japan.. kohgami@med.hokudai.ac.jp
SO Investigative ophthalmology & visual science, (2003 Jun) 44 (6) 2694-701. Journal code: 7703701. ISSN: 0146-0404.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200306
ED Entered STN: 20030604
Last Updated on STN: 20030613
Entered Medline: 20030612
AB PURPOSE: **Astaxanthin** (AST) is a carotenoid that is found in marine animals and vegetables. Several previous studies have demonstrated that **AST** exhibits a wide variety of biological activities including antioxidant, antitumor, and anti-Helicobacter pylori effects. In this study, attention was focused on the antioxidant effect of **AST**. The object of the present study was to investigate the efficacy of **AST** in endotoxin-induced uveitis (EIU) in rats. In addition, the effect of **AST** on endotoxin-induced nitric oxide (NO), prostaglandin E2 (PGE2), and tumor necrosis factor (TNF)-alpha production in a mouse macrophage cell line (RAW 264.7) was studied in vitro. METHODS: EIU was induced in male Lewis rats by a footpad injection of lipopolysaccharide (LPS). **AST** or prednisolone was administered intravenously at 30 minutes before, at the same time as, or at 30 minutes after LPS treatment. The number of infiltrating cells and protein concentration in the aqueous humor collected at 24 hours after LPS treatment was determined. RAW 264.7 cells were pretreated with various concentrations of **AST** for 24 hours and subsequently stimulated with 10 microg/mL of LPS for 24 hours. The levels of PGE2, TNF-alpha, and NO production were determined in vivo and in vitro. RESULTS: **AST** suppressed the development of EIU in a dose-dependent fashion. The anti-inflammatory effect of 100 mg/kg **AST** was as strong as that of 10 mg/kg prednisolone. **AST** also decreased production of NO, activity of inducible nitric oxide **synthase** (NOS), and production of PGE2 and TNF-alpha in RAW264.7 cells in vitro in a dose-dependent manner. CONCLUSIONS: This study suggests that **AST** has a dose-dependent ocular anti-inflammatory effect, by the suppression of NO, PGE2, and TNF-alpha production, through directly blocking NOS enzyme activity.
CT Check Tags: Comparative Study; Male; Support, Non-U.S. Gov't Animals
*Antioxidants: PD, pharmacology
Aqueous Humor: ME, metabolism
Cell Count
Cell Line
Cell Survival
Dinoprostone: BI, biosynthesis
Dose-Response Relationship, Drug
*Lipopolysaccharides: TO, toxicity
Macrophages: DE, drug effects
Macrophages: ME, metabolism
Nitric Oxide: BI, biosynthesis
Nitric-Oxide Synthase: AI, antagonists & inhibitors
Nitric-Oxide Synthase: ME, metabolism

Prednisolone: PD, pharmacology
 Rats
 Rats, Inbred Lew
 *Salmonella typhimurium
 Tumor Necrosis Factor: BI, biosynthesis
 Uveitis, Anterior: CI, chemically induced
 Uveitis, Anterior: ME, metabolism
 *Uveitis, Anterior: PC, prevention & control
 *beta Carotene: AA, analogs & derivatives
 *beta Carotene: PD, pharmacology
 RN 10102-43-9 (Nitric Oxide); 363-24-6 (Dinoprostone); 472-61-7
 (astaxanthine); 50-24-8 (Prednisolone); 7235-40-7 (beta Carotene)
 CN 0 (Antioxidants); 0 (Lipopolysaccharides); 0 (Tumor Necrosis Factor); EC
 1.14.13.- (inducible nitric oxide **synthase**); EC 1.14.13.39
 (Nitric-Oxide **Synthase**)

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E1 1 HOSHINO SYUUJI/AU
 E2 1 HOSHINO SYUZO/AU
 E3 4343 --> HOSHINO T/AU
 E4 5 HOSHINO T */AU
 E5 1 HOSHINO T K/AU
 E6 1 HOSHINO TADA/AU
 E7 1 HOSHINO TADAFUSA/AU
 E8 21 HOSHINO TADAHIDE/AU
 E9 27 HOSHINO TADAHICO/AU
 E10 1 HOSHINO TADAMATSU/AU
 E11 73 HOSHINO TADAO/AU
 E12 55 HOSHINO TADASHI/AU

=> s e3

L12 4343 "HOSHINO T"/AU

=> e ojima k/au

E1 30 OJIMA JUN/AU
 E2 128 OJIMA JURO/AU
 E3 471 --> OJIMA K/AU
 E4 1 OJIMA K T I/AU
 E5 13 OJIMA KAORU/AU
 E6 3 OJIMA KASAHARA S/AU
 E7 1 OJIMA KASAHARA SAKIKO/AU
 E8 1 OJIMA KATSUHIKO/AU
 E9 2 OJIMA KATSUHIRO/AU
 E10 13 OJIMA KATSUJI/AU
 E11 30 OJIMA KAZUMA/AU

E12 1 OJIMA KAZUMO/AU

=> s e3

L13 471 "OJIMA K"/AU

=> e setoguchi y/au

E1 1 SETOGUCHI TSUTOMU/AU

E2 1 SETOGUCHI U M/AU

E3 435 --> SETOGUCHI Y/AU

E4 1 SETOGUCHI Y M/AU

E5 63 SETOGUCHI YASUHIRO/AU

E6 1 SETOGUCHI YOHICHI/AU

E7 1 SETOGUCHI YOICHI/AU

E8 23 SETOGUCHI YOICHI/AU

E9 1 SETOGUCHI YOSHIHIRO/AU

E10 15 SETOGUCHI YOSHIKO/AU

E11 8 SETOGUCHI YOSHINORI/AU

E12 6 SETOGUCHI YOSHIO/AU

=> s e3

L14 435 "SETOGUCHI Y"/AU

=> s l12 or l13 or l14

L15 5227 L12 OR L13 OR L14

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L14 435 S E3

L15 5227 S L12 OR L13 OR L14

=> s l2 and l15

L16 5 L2 AND L15

=> dup rem l16

PROCESSING COMPLETED FOR L16

L17 3 DUP REM L16 (2 DUPLICATES REMOVED)

=> d 1-3 ibib ab

L17 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 2002-11049 BIOTECHDS

TITLE: Production of carotenoids, particularly astaxanthin, useful as colorant in fish feeds, by culturing mutant organism with reduced alternative oxidase activity;

vector plasmid pCR2.1-TOPO-mediated gene transfer and expression in Escherichia coli, DNA primer, antisense and polymerase chain reaction for use in fish feedstuff and as a food-additive and colorant

AUTHOR: HOSHINO T.; OJIMA K.; SETOGUCHI Y
PATENT ASSIGNEE: HOFFMANN LA ROCHE and CO AG F
PATENT INFO: NO 200102552 26 Nov 2001
APPLICATION INFO: NO 2000-2552 24 May 2000
PRIORITY INFO: EP 2000-111148 24 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: Norwegian
OTHER SOURCE: WPI: 2002-194135 [25]

AB DERWENT ABSTRACT:

NOVELTY - Production of carotenoids (I) by growing an organism (A) produced by treating a (I)-producing organism to reduce activity of an alternative oxidase (AO) and selecting strains with increased (I) productivity.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for (1) a method for producing a mutant strain with increased (I) productivity by growing a parent organism under conditions that reduce AO activity, and selecting; (2) mutant strain produced by method (a); (3) recombinant organism with increased (I) productivity, produced by altering expression of the AO gene; and (4) recombinant DNA encoding an AO from a (I)-producing organism.

BIOTECHNOLOGY - Recombinant DNA of (d) is a 3724 bp sequence (2), encoding a 410 amino acid protein (1), from P. rhodozyma, or a sequence with at least 55% identity at the nucleic acid level or 51.5% identity at the protein level. Preparation: Degenerate primers (sequences given) were designed based on sequences conserved in known AO genes and used for polymerase chain reaction (PCR) amplification of cDNA from a spontaneous SHAM-resistant mutant of P. rhodozyma. Amplicons were cloned into pCR2.1-TOPO for expression in Escherichia coli TOP10. One isolated amplicon was used to screen genomic DNA from the same source to select a 6 kb EcoRI insert of which 2561 bp were sequenced to identify a 1206 bp reading frame containing 10 exons and 9 introns. The Genome Walker kit was used to clone the region 5' of the coding sequence to identify a 1406 bp promoter; together with the coding region this forms sequence (2). An antisense sequence corresponding to the entire structural gene of AO was amplified by PCR and cloned under control of the AST gene promoter and terminator. When the recombinant vector was used to transform P. rhodozyma, one clone with increased pigmentation was detected.

USE - The method is particularly used to prepare astaxanthin, used as a fish feed additive to impart an orange-red color to the flesh.

ADVANTAGE - (A) provide yields of (I) up to 50% higher than wild-type strains.

EXAMPLE - When the wild-type Phaffia rhodozyma was grown for 65 hr at 20 degrees C, the content of astaxanthin was 0.211 mg/g dry cells. When the same strain was transformed with a vector that expressed a sequence antisense to the alternative oxidase gene, the corresponding figure was 0.240 mg/g dry cells, i.e. an increase of about 15%.

L17 ANSWER 2 OF 3 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

ACCESSION NUMBER: 95053084 EMBASE
DOCUMENT NUMBER: 1995053084
TITLE: A case of acute carbon tetrachloride poisoning.
AUTHOR: Hoshino T.; Komatsu M.; Ono T.; Funaoka M.; Kato J.; Ishii T.; Kuramitsu T.; Miura K.; Masamune O.; Hojo H.
CORPORATE SOURCE: First Dept. of Internal Medicine, Akita University School of Medicine, Akita, Japan
SOURCE: Acta Hepatologica Japonica, (1994) 35/12 (882-886).
ISSN: 0451-4203 CODEN: KNZOAU
COUNTRY: Japan

DOCUMENT TYPE: Journal; Article
 FILE SEGMENT: 005 General Pathology and Pathological Anatomy
 040 Drug Dependence, Alcohol Abuse and Alcoholism
 048 Gastroenterology
 052 Toxicology
 LANGUAGE: Japanese
 SUMMARY LANGUAGE: English; Japanese
 AB A 55-year-old man who had drunk for past twenty-five years accidentally ingested carbon tetrachloride. He was admitted to a local hospital, complaining of abdominal pain, nausea, vomiting and diarrhea 2 hours after ingestion. He was transferred to our hospital because his condition rapidly deteriorated. Examination on admission revealed mild jaundice and hepatomegaly. Laboratory findings were as follows; AST 5160 IU/l, ALT 3000 IU/l, T. Bil 2.5 mg/dl, PT 35.3%, superoxide dismutase (SOD) 36.2%. Haptoglobin and lipoperoxide were within normal limit. His liver function was normalized at twenty-eight days after admission. Histological findings were as follows; perivenular and pericellular fibrosis, ballooned hepatocytes, infiltration of small round inflammatory cells and polymorphonuclear cells, fat droplets and necrosis of hepatocytes. These changes were dominant in centrilobular area (Zone 3). These findings were compatible to those of acute carbon tetrachloride poisoning superimposed on chronic alcoholic changes and may suggest that hepatotoxicity of carbon tetrachloride is reinforced by alcohol intake.

L17 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 1
 ACCESSION NUMBER: 92405956 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 1382031
 TITLE: Overlap and discrepancy between tests for anti-C100, anti-GOR and anti-CP9 in patients with chronic liver disease and inhabitants in Saga, Japan.
 AUTHOR: Setoguchi Y; Yamamoto K; Ozaki I; Wada I; Hara T; Fujio N; Mizuta T; Sakai T; Nagumo F; Tadano J
 CORPORATE SOURCE: Department of Internal Medicine, Saga Medical School, Japan.
 SOURCE: Gastroenterologia Japonica, (1992 Aug) 27 (4) 502-7. Journal code: 0152744. ISSN: 0435-1339.
 PUB. COUNTRY: Japan
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199210
 ENTRY DATE: Entered STN: 19921106
 Last Updated on STN: 19970203
 Entered Medline: 19921022
 AB The authors evaluated the clinical significance of anti-C100, anti-GOR and anti-CP9 in hepatitis C virus (HCV)-related liver disease in two populations: 459 healthy subjects and 385 patients with chronic liver disease (CLD). Previously we reported high rates of mortality and morbidity (5.3%) of CLD in subjects in Saga, Japan. This was ascribed to the high prevalence (10.8%) of anti-HCV among randomized populations, as detected by the C100 ELISA test system, as compared with a finding of 2-3% in Japanese blood donors in the same decade. The incidence of anti-C100, anti-GOR and anti-CP9 detected by ELISA test system in the healthy population currently surveyed was 17.0%, 19.2% and 32.0% respectively, as compared with 75.3%, 60.3% and 73.0% respectively, in those with CLD. The incidence of positivity for at least one of the three antibodies was high (36.4%) among healthy subjects, and even higher (86.5%) among the patients with CLD. In the healthy subjects, incidence of positivity increased with age. The healthy and CLD populations differed in the proportion of cases positive for all three antibodies vs. those positive for at least one antibody: healthy subjects, 52/167, 31.1%, vs. CLD patients, 197/333, 59.2%; P less than 0.01. Among the anti-C100-positive healthy cases, there was a significantly high level of AST, ALT, ZTT and gamma GTP compared with negative cases, with or without anti-GOR and anti-CP9 (P

less than 0.01-0.05). These observations suggest that the presence of anti-C100 may be related to the active state of HCV-related liver disease. (ABSTRACT TRUNCATED AT 250 WORDS)

=> d his

(FILE 'HOME' ENTERED AT 15:04:15 ON 13 APR 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 15:04:54 ON 13 APR 2004

L1 3 S ASTAXANTHIN (A) SYNTHASE?
L2 19847 S "AST"
L3 5489 S ASTAXANTHIN
L4 36 S L2 AND L3
L5 1238 S RHODOZYMA
L6 6 S L4 AND L5
L7 3 DUP REM L6 (3 DUPLICATES REMOVED)
L8 212 S L2 AND SYNTHASE?
L9 0 S L5 AND L8
L10 4 S L3 AND L8
L11 1 DUP REM L10 (3 DUPLICATES REMOVED)
E HOSHINO T/AU
L12 4343 S E3
E OJIMA K/AU
L13 471 S E3
E SETOGUCHI Y/AU
L14 435 S E3
L15 5227 S L12 OR L13 OR L14
L16 5 S L2 AND L15
L17 3 DUP REM L16 (2 DUPLICATES REMOVED)

=> s l2 (a)gene

L18 66 L2 (A) GENE

=> dup rem l18

PROCESSING COMPLETED FOR L18

L19 28 DUP REM L18 (38 DUPLICATES REMOVED)

=> d 1-28 ibib ab

L19 ANSWER 1 OF 28 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003467793 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 14529747
TITLE: Expression of allatostatins in the Mediterranean field cricket, *Gryllus bimaculatus* de Geer (Ensifera, Gryllidae).
AUTHOR: Meyering-Vos M; Hoffmann K H
CORPORATE SOURCE: Department of Animal Ecology I, University of Bayreuth, Bayreuth 95440, Germany.. Martina-Meyering-Vos@uni-bayreuth.de
SOURCE: Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology, (2003 Oct) 136 (2) 207-15.
Journal code: 9516061. ISSN: 1096-4959.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20031008
Last Updated on STN: 20031218
AB The allatostatin (AST) type A gene of the cricket *Gryllus bimaculatus* encodes a hormone precursor including at least 14 putative peptides with a common C-terminus Y/FXFG/L/amide. By RT-PCR we have analyzed the expression of the allatostatin precursor in various tissues of 0-21 days

old adult virgin and mated females. In 3-day-old virgin females, the gene is strongly expressed in the brain (oesophageal ganglion), the suboesophageal ganglion and the caecum, but to a lower extent in other parts of the digestive tract (ileum, midgut, colon), and in various other tissues such as the fat body, ovaries and female accessory reproductive glands. In the brain and ovaries of virgin females, the AST expression is rather constant throughout adult life, whereas in brains of mated animals, expression is low until day 7, but increases sharply from day 8 onwards to reach values triple those before day 7. In ovaries of mated animals **AST gene** expression is also age-dependent, with high expression rates during the first 4 days after imaginable moult, a second but smaller peak from day 15 to 21, and very low values in between. In the fat body of virgin crickets allatostatin expression is high during the first 9 days after ecdysis and declines thereafter, whereas in mated animals two peak values, day 1 and day 6, are observed, and a third peak in older animals.

L19 ANSWER 2 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2

ACCESSION NUMBER: 2003:488183 BIOSIS
DOCUMENT NUMBER: PREV200300489979
TITLE: Fine mapping of **AST gene** in Arabidopsis.
AUTHOR(S): Mao Ai-Jun; Wang Tai [Reprint Author]; Song Yan-Ru
CORPORATE SOURCE: Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China
twang@ns.ibcas.ac.cn
SOURCE: Acta Botanica Sinica, (January 2003) Vol. 45, No. 1, pp. 88-92. print.
CODEN: CHWHAY. ISSN: 0577-7496.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Oct 2003
Last Updated on STN: 22 Oct 2003

AB The ast (anthocyanin spotted testa) mutant, which was induced by carbon ion radiation, was a single recessive gene mutant of Arabidopsis thaliana (L.) Heynh. with spotted pigment in seed coats, and involved in the anthocyanin biosynthesis. To clone the **AST gene** by map-based cloning strategy, a series of molecular markers were designed according to the SNPs (single nucleotide polymorphisms) and insertion/deletion polymorphisms in the Arabidopsis database. With these molecular markers, the fine-structure mapping of the **AST gene** was finished, the AST locus was located in BAC clone T13M11. It was suggested that the AST candidate gene was T13M11. 8 in the T13M11. This gene was 1 432 bp long with 6 exons and 5 introns. The putative protein of T13M11. 8 gene was similar to dihydroflavonol 4-reductase (DFR), which was an important enzyme in the anthocyanin biosynthesis pathway.

L19 ANSWER 3 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:532320 BIOSIS
DOCUMENT NUMBER: PREV200300534283
TITLE: The carotenoid biosynthesis genes crtI, crtYB and ast of Phaffia rhodozyma are located on the same chromosome.
AUTHOR(S): Echavarri-Erasun, C. [Reprint Author]; Johnson, E. A. [Reprint Author]
CORPORATE SOURCE: University of Wisconsin-Madison, Madison, WI, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2003) Vol. 103, pp. P-132.
<http://www.asmta.org/mtgsrc/generalmeeting.htm>. cd-rom.
Meeting Info.: 103rd American Society for Microbiology General Meeting. Washington, DC, USA. May 18-22, 2003.
American Society for Microbiology.
ISSN: 1060-2011 (ISSN print).

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Nov 2003
Last Updated on STN: 12 Nov 2003

AB Carotenoids are naturally occurring pigments formed through the isoprenoid pathway that impart attractive color to animals and plants and also have essential biological functions as antioxidants, membrane stabilizers and as precursors to essential metabolites such as vitamin A. The food industry utilizes carotenoids as natural sources of pigmentation for enhancing the organoleptic appeal of certain foods, including salmonids and crustacea produced in aquaculture. Carotenoids have also gained attention for their potential health benefits, leading to potential new uses as nutraceuticals. The basidiomycetous yeast *Phaffia rhodozyma* (teleomorph *Xanthophyllomyces dendrorhous*) produces astaxanthin as its major carotenoid and is a commercial source of this important pigment. Although a detailed understanding of carotenoid biosynthesis in *P. rhodozyma* is not yet available, the structural genes *crtI*, *crtYB* and *ast* have recently been reported to code for enzymes involved in the conversion of the colorless precursor carotenoid phytoene to astaxanthin, and their nucleotide sequences are available. We used Pulsed Field Gel Electrophoresis (PFGE) to analyze electrophoretic karyotypes of several wild-type *P. rhodozyma* strains. Southern blots were analyzed with ³²P-labeled DNA probes targeting the *crtI*, *crtYB* and *ast* genes. These experiments revealed that all three genes are located on the same 1.6-1.9 MB chromosome. This represents the first work describing the physical locations of genes involved in astaxanthin biosynthesis in *P. rhodozyma*. Further studies will focus on determining if these genes are clustered and on regulation of their expression.

L19 ANSWER 4 OF 28 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-12745 BIOTECHDS

TITLE: Airway-specific trypsin-like enzymes for use in diagnosis and screening compounds or polypeptides as inhibitors of AST activity, PAR activation and mucus production, and judging therapeutic efficacy;
vector expression in host cell for recombinant protein gene production, antibody, for diagnosis and drug screening

AUTHOR: EGUCHI H; CHOKKI M; YAMAMURA S; MITA R; MASEGI T
PATENT ASSIGNEE: TEIJIN LTD
PATENT INFO: WO 2002018562 7 Mar 2002
APPLICATION INFO: WO 2000-JP7349 28 Aug 2000
PRIORITY INFO: JP 2001-59753 5 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2002-315539 [35]

AB DERWENT ABSTRACT:

NOVELTY - An airway-specific trypsin-like enzyme (AST) is a protein comprising the whole or a part of the amino acid sequence of (I), in which a propeptide moiety containing all or part of an amino acid sequence of AST between Met at position 1 and Arg at position 186 is bonded to a trypsin-like moiety containing Ile at position 187 to Ile at position 418 in a 533 amino acid sequence via a disulfide bond.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an AST in a mammalian counterpart protein having not less than 66 % homology with the AST of sequence (I), in which a part equivalent to the propeptide moiety is bonded to a part equivalent to the trypsin-like moiety via a disulfide bond; (2) detecting a test compound or polypeptide with inhibitory activity on AST by mixing an enzyme substrate and the test compound or polypeptide before incubating with the AST or cells or tissues expressing the AST to react with the substrate and assaying the reaction product; (3) detecting a test compound or polypeptide with inhibitory activity on protease-activating receptor

signal transduction pathway activation by AST by mixing with the AST or cells or tissues expressing the AST, and then cells expressing protease-activating receptor, and using the activation as indication; (4) evaluating inhibitory activity of a test compound or polypeptide on calcium flow into cells by AST by using intracellular calcium concentration as indication; (5) evaluating inhibitory activity of a test compound or polypeptide on interleukin (IL)-8 freeing promoting effect by AST by using the free amount or expression dose of IL-8 or promoter transcription activity as indication; (6) detecting inhibitory activity of a test compound or polypeptide on mucus production promotion by mixing with the AST or cells or tissues expressing the enzyme and cells capable of producing mucus, and using mucus production capability as indication; (7) detecting inhibitory activity of a test compound or polypeptide on production of the EGFR pathway activation by mixing with the AST or cells or tissues expressing the enzyme and cells with EGFR signal transduction system, and using EGFR signal transduction system activation as indication; (8) a gene encoding the AST, or mRNA or cDNA originating from the gene; (9) an expression vector of the AST produced by using the whole or a part of the gene, mRNA or cDNA; (10) cells transfected or transformed with the expression vector; (11) producing the AST by culturing the cells and then purifying the product from the culture liquor; (12) detecting expression dose of a nucleic acid corresponding to the **AST gene** by using a whole or part of the already specified gene, mRNA or cDNA; (13) screening compounds or polypeptides with inhibitory activity on the AST, or inhibitors of the enzyme activation, or their therapeutic efficacy by administering the test compound or polypeptide to mammals with a disease due to excessive activation or up-regulation of the AST, followed by judging the effect; (14) screening inhibitors of PAR activation due the AST or judging their therapeutic efficacy by administering the test compound or polypeptide to mammals with a disease due to excessive activation or up-regulation of the AST, followed by judging the effect; (15) screening inhibitors of mucus production promoters or judging their therapeutic efficacy by administering the test compound or polypeptide to mammals with a disease due to excessive activation or up-regulation of the AST, followed by judging the effect; (16) screening inhibitors of EGFR pathway activation due to the AST or judging their therapeutic efficacy by administering the test compound or polypeptide to mammals with a disease due to excessive activation or up-regulation of the AST, followed by judging the effect; (17) an antibody that can specifically bind with the AST; (18) a monoclonal antibody that can specifically bind with the AST; (19) a monoclonal antibody that can inhibit the AST, and/or can inhibit its activation; and (20) detecting or assaying the AST by using the antibody.

USE - The enzymes are or use in diagnosis and screening compounds or polypeptides as inhibitors of AST activity, PAR activation, mucus production promotion, cell proliferation, calcium flow into cells or EGFR pathway activation by AST and judging therapeutic efficacy (claimed).

EXAMPLE - From sputum, active natural AST was prepared, e.g. by the method of Yamaoka (J. Biol. Chemical, 1998, volume 273, p. 11895). Recombinant ASTs were also obtained for subsequent studies. (165 pages)

L19 ANSWER 5 OF 28	MEDLINE on STN	DUPLICATE 3
ACCESSION NUMBER:	2002164196 MEDLINE	
DOCUMENT NUMBER:	PubMed ID: 11895956	
TITLE:	Role of various enterotoxins in Aeromonas hydrophila-induced gastroenteritis: generation of enterotoxin gene-deficient mutants and evaluation of their enterotoxic activity.	
AUTHOR:	Sha Jian; Kozlova E V; Chopra A K	
CORPORATE SOURCE:	Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, Texas 77555-1070, USA.	
CONTRACT NUMBER:	AI41611 (NIAID)	
SOURCE:	Infection and immunity, (2002 Apr) 70 (4) 1924-35. Journal code: 0246127. ISSN: 0019-9567.	

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 20020317
Last Updated on STN: 20020412
Entered Medline: 20020411

AB Three enterotoxins from the *Aeromonas hydrophila* diarrheal isolate SSU have been molecularly characterized in our laboratory. One of these enterotoxins is cytotoxic in nature, whereas the other two are cytotoxic enterotoxins, one of them heat labile and the other heat stable. Earlier, by developing an isogenic mutant, we demonstrated the role of a cytotoxic enterotoxin in causing systemic infection in mice. In the present study, we evaluated the role of these three enterotoxins in evoking diarrhea in a murine model by developing various combinations of enterotoxin gene-deficient mutants by marker-exchange mutagenesis. A total of six isogenic mutants were prepared in a cytotoxic enterotoxin gene (act)-positive or -negative background strain of *A. hydrophila*. We developed two single knockouts with truncation in either the heat-labile (alt) or the heat-stable (ast) cytotoxic enterotoxin gene; three double knockouts with truncations of genes encoding (i) alt and ast, (ii) act and alt, and (iii) act and ast genes; and a triple-knockout mutant with truncation in all three genes, act, alt, and ast. The identity of these isogenic mutants developed by double-crossover homologous recombination was confirmed by Southern blot analysis. Northern and Western blot analyses revealed that the expression of different enterotoxin genes in the mutants was correspondingly abrogated. We tested the biological activity of these mutants in a diet-restricted and antibiotic-treated mouse model with a ligated ileal loop assay. Our data indicated that all of these mutants had significantly reduced capacity to evoke fluid secretion compared to that of wild-type *A. hydrophila*; the triple-knockout mutant failed to induce any detectable level of fluid secretion. The biological activity of selected *A. hydrophila* mutants was restored after complementation. Taken together, we have established a role for three enterotoxins in *A. hydrophila*-induced gastroenteritis in a mouse model with the greatest contribution from the cytotoxic enterotoxin Act, followed by the Alt and Ast cytotoxic enterotoxins.

L19 ANSWER 6 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:11206 BIOSIS
DOCUMENT NUMBER: PREV200300011206
TITLE: Wiring the zebrafish: Axon guidance and synaptogenesis.
AUTHOR(S): Hutson, Lara D. [Reprint Author]; Chien, Chi-Bin [Reprint Author]
CORPORATE SOURCE: Department of Neurobiology and Anatomy, University of Utah Medical Center, 20 North 1900 East, Room 401 Medical Research and Engineering Building, Salt Lake City, UT, 84132, USA
chi-bin.chien@hsc.utah.edu
SOURCE: Current Opinion in Neurobiology, (February 2002) Vol. 12, No. 1, pp. 87-92. print.
ISSN: 0959-4388.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 18 Dec 2002
Last Updated on STN: 18 Dec 2002

L19 ANSWER 7 OF 28 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-11049 BIOTECHDS
TITLE: Production of carotenoids, particularly astaxanthin, useful as colorant in fish feeds, by culturing mutant organism with

reduced alternative oxidase activity;
vector plasmid pCR2.1-TOPO-mediated gene transfer and
expression in Escherichia coli, DNA primer, antisense and
polymerase chain reaction for use in fish feedstuff and as
a food-additive and colorant

AUTHOR: HOSHINO T; OJIMA K; SETOGUCHI Y
PATENT ASSIGNEE: HOFFMANN LA ROCHE and CO AG F
PATENT INFO: NO 200102552 26 Nov 2001
APPLICATION INFO: NO 2000-2552 24 May 2000
PRIORITY INFO: EP 2000-111148 24 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: Norwegian
OTHER SOURCE: WPI: 2002-194135 [25]

AB DERWENT ABSTRACT:

NOVELTY - Production of carotenoids (I) by growing an organism (A) produced by treating a (I)-producing organism to reduce activity of an alternative oxidase (AO) and selecting strains with increased (I) productivity.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for (1) a method for producing a mutant strain with increased (I) productivity by growing a parent organism under conditions that reduce AO activity, and selecting; (2) mutant strain produced by method (a); (3) recombinant organism with increased (I) productivity, produced by altering expression of the AO gene; and (4) recombinant DNA encoding an AO from a (I)-producing organism.

BIOTECHNOLOGY - Recombinant DNA of (d) is a 3724 bp sequence (2), encoding a 410 amino acid protein (1), from *P. rhodozyma*, or a sequence with at least 55% identity at the nucleic acid level or 51.5% identity at the protein level. Preparation: Degenerate primers (sequences given) were designed based on sequences conserved in known AO genes and used for polymerase chain reaction (PCR) amplification of cDNA from a spontaneous SHAM-resistant mutant of *P. rhodozyma*. Amplicons were cloned into pCR2.1-TOPO for expression in *Escherichia coli* TOP10. One isolated amplicon was used to screen genomic DNA from the same source to select a 6 kb EcoRI insert of which 2561 bp were sequenced to identify a 1206 bp reading frame containing 10 exons and 9 introns. The Genome Walker kit was used to clone the region 5' of the coding sequence to identify a 1406 bp promoter; together with the coding region this forms sequence (2). An antisense sequence corresponding to the entire structural gene of AO was amplified by PCR and cloned under control of the **AST** gene promoter and terminator. When the recombinant vector was used to transform *P. rhodozyma*, one clone with increased pigmentation was detected.

USE - The method is particularly used to prepare astaxanthin, used as a fish feed additive to impart an orange-red color to the flesh.

ADVANTAGE - (A) provide yields of (I) up to 50% higher than wild-type strains.

EXAMPLE - When the wild-type *Phaffia rhodozyma* was grown for 65 hr at 20 degrees C, the content of astaxanthin was 0.211 mg/g dry cells. When the same strain was transformed with a vector that expressed a sequence antisense to the alternative oxidase gene, the corresponding figure was 0.240 mg/g dry cells, i.e. an increase of about 15%.

L19 ANSWER 8 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:50159 HCAPLUS

DOCUMENT NUMBER: 134:126819

TITLE: Human anion transporter gene implicated in salla disease and lysosomal sialic acid transport and its therapeutic and diagnostic uses

PATENT ASSIGNEE(S): Akzo Nobel N.V., Neth.

SOURCE: Eur. Pat. Appl., 21 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1069184	A1	20010117	EP 1999-202341	19990716
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: EP 1999-202341 19990716

AB This invention describes a novel human sialic acid transporter derived from EST. This invention also encompasses nucleic acids encoding this protein. The protein can be used in the identification of activators or inhibitors. Several mutations have been observed in patients suffering from genetic disorders such as lysosomal storage disorders (Salla disease (SD) and the more severe infantile type ISSD). These mutations are informative in a diagnostic context.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 9 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:397643 BIOSIS

DOCUMENT NUMBER: PREV200100397643

TITLE: Differences in levels of secreted locus of enterocyte effacement proteins between human disease-associated and bovine *Escherichia coli* O157.

AUTHOR(S): McNally, Alan; Roe, Andrew J.; Simpson, Sally; Thomson-Carter, Fiona M.; Hoey, D. E. Elaine; Currie, Carol; Chakraborty, Trinad; Smith, David G. E.; Gally, David L. [Reprint author]

CORPORATE SOURCE: Department of Veterinary Pathology, ZAP Laboratory, Teviot Place, Edinburgh, EH8 9AG, UK
dgally@ed.ac.uk

SOURCE: Infection and Immunity, (August, 2001) Vol. 69, No. 8, pp. 5107-5114. print.

CODEN: INFIBR. ISSN: 0019-9567.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 22 Aug 2001

Last Updated on STN: 22 Feb 2002

AB Ongoing extensive epidemiological studies of verotoxin-carrying *Escherichia coli* O157 (stx+ eae+) have shown this bacterial pathogen to be common in cattle herds in the United States and the United Kingdom. However, the incidence of disease in humans due to this pathogen is still very low. This study set out to investigate if there is a difference between strains isolated from human disease cases and those isolated from asymptomatic cattle which would account for the low disease incidence of such a ubiquitous organism. The work presented here has compared human disease strains from both sporadic and outbreak cases with a cross-section, as defined by pulsed-field gel electrophoresis, of *E. coli* O157 strains from cattle. Human (n = 22) and bovine (n = 31) strains were genotyped for carriage of the genes for Shiga-like toxin types 1, 2, and 2c; *E. coli* secreted protein genes espA, espB, and espP; the enterohemolysin gene; eae (intimin); ast (enteroaggregative *E. coli* stable toxin (EAST)); and genes for common *E. coli* adhesins. Strains were also phenotyped for hemolysin, EspP, Tir, and EspD expression as well as production of actin and cytoskeletal rearrangement associated with attaching and effacing (A/E) lesions on HeLa cells. The genotyping confirmed that there was little difference between the two groups, including carriage of stx2 and stx2c, which was similar in both sets. ast alleles were confirmed to all contain mutations that would prevent EAST expression. espP mutations were found only in cattle strains (5 of 30). Clear differences were observed in the expression of locus of enterocyte effacement (LEE)-encoded factors between strains and in different media. EspD, as an indicator of LEE4 (espA, -B, and -D) expression, and Tir

levels in supernatants were measured. Virtually all strains from both sources could produce EspD in Luria-Bertani broth, although at very different levels. Standard trichloroacetic acid precipitation of secreted proteins from tissue culture medium produced detectable levels of EspD from the majority of strains of human origin (15 of 20) compared with only a few (4 of 20) bovine strains ($P < 0.001$), which is indicative of much higher levels of protein secretion from the human strains. Addition of bovine serum albumin carrier protein before precipitation and enhanced detection techniques confirmed that EspD could be detected after growth in tissue culture medium for all strains, but levels from strains of human origin were on average 90-fold higher than those from strains of bovine origin. In general, levels of secretion also correlated with ability to form A/E lesions on HeLa cells, with only the high-level protein secretors in tissue culture medium exhibiting a localized adherence phenotype. This research shows significant differences between human- and bovine-derived *E. coli* O157 (stx+ eae+) strains and their production of certain LEE-encoded virulence factors. These data support the recent finding of Kim et al. (J. Kim, J. Nietfeldt, and A. K. Benson, Proc. Natl. Acad. Sci. USA 96:13288-13293, 1999) proposing different *E. coli* O157 lineages in cattle and humans and extend the differential to the regulation of virulence factors. Potentially only a subset of *E. coli* O157 isolates (stx+ eae+) in cattle may be capable of causing severe disease in humans.

L19 ANSWER 10 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:303509 HCAPLUS

DOCUMENT NUMBER: 135:17048

TITLE: Astray, a zebrafish roundabout homolog required for retinal axon guidance

AUTHOR(S): Fricke, Cornelia; Lee, Jeong-Soo; Geiger-Rudolph, Silke; Bonhoeffer, Friedrich; Chien, Chi-Bin

CORPORATE SOURCE: Department of Neurobiology and Anatomy, University of Utah Medical Center, Salt Lake City, UT, 84132, USA

SOURCE: Science (Washington, DC, United States) (2001), 292(5516), 507-510

CODEN: SCIEAS; ISSN: 0036-8075

PUBLISHER: American Association for the Advancement of Science

DOCUMENT TYPE: Journal

LANGUAGE: English

AB As growing retinotectal axons navigate from the eye to the tectum, they sense guidance mols. distributed along the optic pathway. Mutations in the zebrafish astray gene severely disrupt retinal axon guidance, causing anterior-posterior pathfinding defects, excessive midline crossing, and defasciculation of the retinal projection. Eye transplantation expts. show that astray function is required in the eye. The authors identify astray as zebrafish robo2, a member of the Roundabout family of axon guidance receptors. Retinal ganglion cells express robo2 as they extend axons. Thus, robo2 is required for multiple axon guidance decisions during establishment of the vertebrate visual projection.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 11 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:502027 BIOSIS

DOCUMENT NUMBER: PREV200100502027

TITLE: Angiotensinogen M235T polymorphism and exercise hemodynamics in postmenopausal women.

AUTHOR(S): McCole, S. D. [Reprint author]; Brown, M. D.; Moore, G. E.; Ferrell, R. E.; Wilund, K. R.; Hagberg, J. M.

CORPORATE SOURCE: University of Wisconsin-Milwaukee, Milwaukee, WI, USA

SOURCE: Medicine and Science in Sports and Exercise, (May, 2001) Vol. 33, No. 5 Supplement, pp. S322. print. Meeting Info.: 48th Annual Meeting of the American College of Sports Medicine. Baltimore, Maryland, USA. May 30-June

02, 2001.
CODEN: MSPEDA. ISSN: 0195-9131.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Oct 2001
Last Updated on STN: 23 Feb 2002

L19 ANSWER 12 OF 28 LIFESCI COPYRIGHT 2004 CSA on STN

ACCESSION NUMBER: 2002:49884 LIFESCI

TITLE: Identification, cloning and characterisation of a
HMW-glutenin gene from an old Hungarian wheat variety,
Bankuti 1201

AUTHOR: Juhasz, A.; Tamas, L.; Karsai, I.; Vida, G.; Lang, L.;
Bed[dblac], Z.

CORPORATE SOURCE: Agricultural Research Institute of the Hungarian Academy of
Sciences, Martonvasar, Hungary

SOURCE: Euphytica, (20010500) vol. 119, no. 1-2, pp. 75-79.
ISSN: 0014-2336.

DOCUMENT TYPE: Journal

FILE SEGMENT: G

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Despite its good functional properties, the variety Bankuti 1201 has been
found to possess 2 + 12 or 3 + 12 allele composition on chromosome 1D. In
order to determine the reason for its quality traits a gene-specific PCR
technique was applied in preliminary experiments to examine the HMW
glutenin allele composition of the variety. In the course of the analysis
a fragment characteristic of Bankuti 1201 was identified and the
nucleotide sequence was determined. This showed the presence of a 1Ax2
super(*) gene variant which, despite near
homology, differed from the original 1Ax2 super(*)
gene at one important point. At 1181 bp of the 1Ax2 super(*)
sequence nucleotide exchange was observed which is the middle nucleotide
of the TCT-TGT base triplet, involving the exchange of serine for
cysteine. The gene was designated 1Ax2 super(*B). The presence of an
extra sulphydryl group, like that of the extra cysteine in the 1Dx5 gene,
facilitates the formation of further disulphide bonds, might lead to an
improvement in gluten quality characters.

L19 ANSWER 13 OF 28 MEDLINE on STN

DUPLICATE 4

ACCESSION NUMBER: 2001024586 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11015403

TITLE: Prevalence of enterotoxin genes in Aeromonas spp. isolated
from children with diarrhea, healthy controls, and the
environment.

AUTHOR: Albert M J; Ansaruzzaman M; Talukder K A; Chopra A K; Kuhn
I; Rahman M; Faruque A S; Islam M S; Sack R B; Mollby R

CORPORATE SOURCE: International Centre for Diarrhoeal Disease Research,
Bangladesh, Dhaka-1000, Bangladesh.. john@hsc.kuniv.edu.kw

CONTRACT NUMBER: RO1A41611

SOURCE: Journal of clinical microbiology, (2000 Oct) 38 (10)
3785-90.

Journal code: 7505564. ISSN: 0095-1137.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200011

ENTRY DATE: Entered STN: 20010322

Last Updated on STN: 20010322

Entered Medline: 20001116

AB Aeromonads are causative agents of a number of human infections. Even

though aeromonads have been isolated from patients suffering from diarrhea, their etiological role in gastroenteritis is unclear. In spite of a number of virulence factors produced by *Aeromonas* species, their association with diarrhea has not been clearly linked. Recently, we have characterized a heat-labile cytotoxic enterotoxin (Alt), a heat-stable cytotoxic enterotoxin (Ast), and a cytotoxic enterotoxin (Act) from a diarrheal isolate of *Aeromonas hydrophila*. Alt and Ast are novel enterotoxins which are not related to cholera toxin; Act is aerolysin related and has hemolytic, cytotoxic, and enterotoxic activities. We studied the distribution of the alt, ast, and act enterotoxin genes in 115 of 125 aeromonads isolated from 1,735 children with diarrhea, in all 27 aeromonads isolated from 830 control children ($P = 7 \times 10^{-4}$ for comparison of rates of isolation of aeromonads from cases versus those from controls), and in 120 randomly selected aeromonads from different components of surface water in Bangladesh. *Aeromonas* isolates which were positive only for the presence of the alt gene had similar distributions in the three sources; the number of isolates positive only for the presence of the ast gene was significantly higher for the environmental samples than for samples from diarrheal children; and isolates positive only for the presence of the act gene were not found in any of the three sources. Importantly, the number of isolates positive for both the alt and ast genes was significantly higher for diarrheal children than for control children and the environment. Thus, this is the first study to indicate that the products of both the alt and ast genes may synergistically act to induce severe diarrhea. In 26 patients, *Aeromonas* spp. were isolated as the sole enteropathogen. Analysis of clinical data from 11 of these patients suggested that isolates positive for both the alt and ast genes were associated with watery diarrhea but that isolates positive only for the alt gene were associated with loose stools. Most of the isolates from the three sources could be classified into seven phenospecies and eight hybridization groups. For the first time, *Aeromonas eucrenophila* was isolated from two children, one with diarrhea and another without diarrhea.

L19 ANSWER 14 OF 28 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
 ACCESSION NUMBER: 2001-05747 BIOTECHDS
 TITLE: Purification, cloning, and characterization of an
 arylsulfotransferase from the anaerobic bacterium *Eubacterium*
rectale IIIH;
 arylsulfotransferase purification, cloning, and
 characterization
 AUTHOR: Goldberg S L; Nanduri V; Cino P M; Patel R
 CORPORATE SOURCE: Bristol-Squibb
 LOCATION: Bristol-Myers Squibb Co. One Squibb Drive, Building 98, New
 Brunswick, NJ 08903, USA.
 SOURCE: J.Ind.Microbiol.Biotechnol.; (2000) 25, 6, 305-09
 CODEN: JIMIE7
 ISSN: 1367-5435
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB. Purification, cloning and characterization of an arylsulfotransferase
 (ASST) from the anaerobic bacterium *Eubacterium rectale* IIIH, was
 studied. Loopful of human feces was suspended in 10 ml BBL chopped meat
 broth and serial 10-fold dilutions were made in the same medium. *E.*
rectale IIIH chromosomal DNA was prepared from cultures grown in potato
 yeast glucose medium, except the cells were treated with 10 mg/ml of
 lysozyme (EC-3.2.1.17) at 37 deg for 15 min before proceeding with cell
 lysis. Restriction fragment used for subcloning into plasmid vectors
 pET5b or plasmid vector pSE380 were obtained from lambdaZAPII clone
 expressing ASST activity or from a plasmid containing the subcloned
 ast gene. Two sequences related to the *E. rectale* ASST
 were obtained from database search. The amino acid sequence of the *E.*
rectale IIIH astA gene was compared to ASSTs from *Klebsiella* A-44 and

Enterobacter amnigenus AR-37. These genes showed only modest homology to Eubacterium version (44.35% similarity and 33.48% identity). Another significant difference between the Klebsiella, Enterobacter and E. rectale ASSTs was the location of the protein in the respective host bacteria. (24 ref)

L19 ANSWER 15 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:479220 BIOSIS
DOCUMENT NUMBER: PREV200000479220
TITLE: Molecular characterization of allatostatin cDNA in central nervous system and midgut from the german cockroach *Blattella germanica*.
AUTHOR(S): Yang, Jae Hyuck; Park, Joong-Cheol; Lee, Bong Hee [Reprint author]
CORPORATE SOURCE: Department of Biology, Korea University, Seoul, 136-701, South Korea
SOURCE: Korean Journal of Entomology, (June, 2000) Vol. 30, No. 2, pp. 131-137. print.
ISSN: 1011-9493.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Nov 2000
Last Updated on STN: 10 Jan 2002

AB This study has been carried out to determine molecular characteristics of *Blattella germanica* allatostatin (AST) inhibiting juvenile hormone (JH) biosynthesis by corpora allata (CA). In order to determine base sequence of coding region in **AST gene**, a cDNA library was constructed by RT-PCR with total RNA purified from both central nervous system (CNS) and midgut of *B. germanica*. To get PCR products, PCR and 5'- and 3'-RACE were conducted using degenerated primers designed from the highly conserved region of **AST genes** from *Diploptera punctata* and *Periplaneta americana*. The amplified DNA fragments were subcloned into pGEM-T Easy vector and three cDNA clones obtained were sequenced. They were all composed of 1,506 bp which are able to encode. 375 amino acids for synthesis of ASTs in the CNS and midgut of the adult german cockroach. The nucleotide sequence of AST cDNA obtained from *B. germanica* shows similarities of 83% and 77% to those from *D. punctata* and *P. americana*, respectively.

L19 ANSWER 16 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:203997 BIOSIS
DOCUMENT NUMBER: PREV199900203997
TITLE: Role of ArgR in activation of the ast operon, encoding enzymes of the arginine succinyltransferase pathway in *Salmonella typhimurium*.
AUTHOR(S): Lu, Chung-Dar; Abdelal, Ahmed T. [Reprint author]
CORPORATE SOURCE: Georgia State University, Dean's Office, Atlanta, GA, 30302-4038, USA
SOURCE: Journal of Bacteriology, (March, 1999) Vol. 181, No. 6, pp. 1934-1938. print.
CODEN: JOBAAAY. ISSN: 0021-9193.
DOCUMENT TYPE: Article
LANGUAGE: English
OTHER SOURCE: Genbank-AF108767
ENTRY DATE: Entered STN: 26 May 1999
Last Updated on STN: 26 May 1999

AB The ast operon, encoding enzymes of the arginine succinyltransferase (AST) pathway, was cloned from *Salmonella typhimurium*, and the nucleotide sequence for the upstream flanking region was determined. The control region contains several regulatory consensus sequences, including binding sites for NtrC, cyclic AMP receptor protein (CRP), and ArgR. The results of DNase I footprintings and gel retardation experiments confirm binding of these regulatory proteins to the identified sites. Exogenous arginine induced AST under nitrogen-limiting conditions, and this induction was

abolished in an argR derivative. AST was also induced under carbon starvation conditions; this induction required functional CRP as well as functional ArgR. The combined data are consistent with the hypothesis that binding of one or more ArgR molecules to a region between the upstream binding sites for NtrC and CRP and two putative promoters plays a pivotal role in modulating expression of the ast operon in response to nitrogen or carbon limitation.

L19 ANSWER 17 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:62064 BIOSIS

DOCUMENT NUMBER: PREV200000062064

TITLE: A new gene, encoding an anion transporter, is mutated in sialic acid storage diseases.

AUTHOR(S): Verheijen, Frans W. [Reprint author]; Verbeek, Elly; Aula, Nina; Beerens, Cecile E. M. T.; Havelaar, Adrie C.; Joosse, Marijke; Peltonen, Leena; Aula, Pertti; Galjaard, Hans; van der Spek, Peter J.; Mancini, Grazia M. S.

CORPORATE SOURCE: Department of Clinical Genetics, Erasmus University and Academic Hospital, Erasmus Medical Centre of Rotterdam, Rotterdam, Netherlands

SOURCE: Nature Genetics, (Dec., 1999) Vol. 23, No. 4, pp. 462-465. print.

ISSN: 1061-4036.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 9 Feb 2000

Last Updated on STN: 3 Jan 2002

AB Sialic acid storage diseases (SASD, MIM 269920) are autosomal recessive neurodegenerative disorders that may present as a severe infantile form (ISSD) or a slowly progressive adult form, which is prevalent in Finland (Salla disease). The main symptoms are hypotonia, cerebellar ataxia and mental retardation; visceromegaly and coarse features are also present in infantile cases. Progressive cerebellar atrophy and dysmyelination have been documented by magnetic resonance imaging (reference 4). Enlarged lysosomes are seen on electron microscopic studies and patients excrete large amounts of free sialic acid in urine. A H⁺/anionic sugar symporter mechanism for sialic acid and glucuronic acid is impaired in lysosomal membranes from Salla and ISSD patients. The locus for Salla disease was assigned to a region of approximately 200 kb on chromosome 6q14-q15 in a linkage study using Finnish families. Salla disease and ISSD were further shown to be allelic disorders. A physical map with P1 and PAC clones was constructed to cover the 200-kb area flanked by the loci D6S280 and D6S1622, providing the basis for precise physical positioning of the gene. Here we describe a new gene, SLC17A5 (also known as AST), encoding a protein (sialin) with a predicted transport function that belongs to a family of anion/cation symporters (ACS). We found a homozygous SLC17A5 mutation (R39C) in five Finnish patients with Salla disease and six different SLC17A5 mutations in six ISSD patients of different ethnic origins. Our observations suggest that mutations in SLC17A5 are the primary cause of lysosomal sialic acid storage diseases.

L19 ANSWER 18 OF 28 LIFESCI COPYRIGHT 2004 CSA on STN

ACCESSION NUMBER: 1998:114971 LIFESCI

TITLE: Arginine catabolism and the arginine succinyltransferase pathway in Escherichia coli

AUTHOR: Schneider, B.L.; Kiupakis, A.K.; Reitzer, L.J.*

CORPORATE SOURCE: Department of Molecular and Cell Biology, Mail Station FO 3.1, The University of Texas at Dallas, P.O. Box 830688, Richardson, TX 75083-0688, USA

SOURCE: J. Bacteriol., (19980800) vol. 180, no. 16, pp. 4278-4286. ISSN: 0021-9193.

DOCUMENT TYPE: Journal

FILE SEGMENT: J

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Arginine catabolism produces ammonia without transferring nitrogen to another compound, yet the only known pathway of arginine catabolism in *Escherichia coli* (through arginine decarboxylase) does not produce ammonia. Our aims were to find the ammonia-producing pathway of arginine catabolism in *E. coli* and to examine its function. We showed that the only previously described pathway of arginine catabolism, which does not produce ammonia, accounted for only 3% of the arginine consumed. A search for another arginine catabolic pathway led to discovery of the ammonia-producing arginine succinyltransferase (AST) pathway in *E. coli*. Nitrogen limitation induced this pathway in both *E. coli* and *Klebsiella aerogenes*, but the mechanisms of activation clearly differed in these two organisms. We identified the *E. coli* gene for succinylornithine aminotransferase, the third enzyme of the AST pathway, which appears to be the first of an *astCADBE* operon. Its disruption prevented arginine catabolism, impaired ornithine utilization, and affected the synthesis of all the enzymes of the AST pathway. Disruption of *astB* eliminated succinylarginine dihydrolase activity and prevented arginine utilization but did not impair ornithine catabolism. Overproduction of AST enzymes resulted in faster growth with arginine and aspartate. We conclude that the AST pathway is necessary for aerobic arginine catabolism in *E. coli* and that at least one enzyme of this pathway contributes to ornithine catabolism.

L19 ANSWER 19 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:85927 HCAPLUS

DOCUMENT NUMBER: 130:307186

TITLE: Identification of genes encoding *Schistosoma mansoni*

antigens using an antigenic sequence tag strategy

AUTHOR(S): Zouain, C. S.; Azevedo, V. A.; Franco, G. R.; Pena, S. D. J.; Goes, A. M.

CORPORATE SOURCE: Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas

Gerais, Belo Horizonte, CEP 30161-970, Brazil

SOURCE: Journal of Parasitology (1998), 84(6), 1307-1310

CODEN: JOPAA2; ISSN: 0022-3395

PUBLISHER: American Society of Parasitologists

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Another approach for the identification of genes that code for antigenic products is described using an antigenic sequence tag (AST) strategy. A *Schistosoma mansoni* adult worm cDNA library was screened with affinity chromatog.-purified Igs from infected human sera and a mild oxidation treatment with sodium periodate. From 1 or both ends of 30 cDNA clones, 30 ASTs were obtained. Of these, 22 were previously known Sm antigens. One clone had matches with entries for other organisms in the databases and 6 had homol. with Sm-expressed sequence tags (EST) entries. These clones, together with another 1 that had no significant database matches, were considered new antigenic genes in *S. mansoni*. The strategy proved to be efficient for the identification of genes that could be used for immunol. studies and evaluation as vaccine candidates.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 20 OF 28 MEDLINE on STN

DUPLICATE 5

ACCESSION NUMBER: 1998053840 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9393691

TITLE: Cloning and characterization of the *aru* genes encoding enzymes of the catabolic arginine succinyltransferase pathway in *Pseudomonas aeruginosa*.

AUTHOR: Itoh Y

CORPORATE SOURCE: Division of Applied Microbiology, National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan.. yosifumi@nfri.affrc.go.jp

SOURCE: Journal of bacteriology, (1997 Dec) 179 (23) 7280-90.
 Journal code: 2985120R. ISSN: 0021-9193.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-AF011922
 ENTRY MONTH: 199712
 ENTRY DATE: Entered STN: 19980116
 Last Updated on STN: 20000303
 Entered Medline: 19971230

AB The arginine succinyltransferase (AST) pathway is the major arginine and ornithine utilization (aru) pathway under aerobic conditions in *Pseudomonas aeruginosa*. A 26-kb DNA fragment of the *P. aeruginosa* PAO1 chromosome carrying the regulatory *argR* gene and the *aru* structural gene cluster was cloned. Complementation tests and nucleotide sequence data established the locations of the *argR*, *aruC*, *aruF*, *aruG*, *aruD*, *aruB*, and *aruE* genes, in that order. The *aruR*, *aruC*, *aruD*, *aruB*, and *aruE* genes specify the ArgR regulatory protein, N2-succinylornithine 5-aminotransferase, N-succinylglutamate 5-semialdehyde dehydrogenase, N2-succinylarginine dihydrolase, and N-succinylglutamate desuccinylase, respectively, and the *aruF* and *aruG* genes encode the subunits (AruAI and AruAII) of arginine and ornithine N2-succinyltransferases. Furthermore, in vivo analysis of transcriptional *aru* fusions and of polar insertion mutations located at different sites in the *aru* cluster indicated the presence of three transcriptional units which are controlled by ArgR. The *aruCFGDB* genes appear to form an operon transcribed from a promoter upstream of *aruC*, whereas *aruE* has its own promoter. The *argR* gene, which is located upstream of the *aruCFGDB* operon, is a member of another (*aot*) operon coding for arginine transport genes. The deduced amino acid sequences of the AST enzymes were compared to those of homologous proteins of *Escherichia coli* specified by the **ast genes** lying in the chromosome region from 39.2 to 39.5 min (Kohara clone 327; GenBank/EMBL/DDJB accession number D90818). The overall organization of the *aru* and **ast genes** in both organisms is similar, with the exception that *E. coli* appears to have a single **AST gene**.

L19 ANSWER 21 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
 ACCESSION NUMBER: 1997:488685 BIOSIS
 DOCUMENT NUMBER: PREV199799787888
 TITLE: Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable:H10 associated with a massive outbreak of gastrointestinal illness.
 AUTHOR(S): Itoh, Yoshihiro [Reprint author]; Nagano, Isao; Kunishima, Miyuki; Ezaki, Takayuki
 CORPORATE SOURCE: Dep. Microbiol., Gifu Univ. Sch. Med., Tsukasa-machi 40, Gifu 500, Japan
 SOURCE: Journal of Clinical Microbiology, (1997) Vol. 35, No. 10, pp. 2546-2550.
 CODEN: JCMIDW. ISSN: 0095-1137.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 ENTRY DATE: Entered STN: 7 Nov 1997
 Last Updated on STN: 7 Nov 1997

AB A massive outbreak of gastrointestinal illness occurred in Tajimi city, Gifu prefecture, in June of 1993 in which 2,697 children in elementary and junior high schools developed severe diarrhea. Stool specimens from 30 children with severe protracted diarrhea were studied. Twenty-seven strains of enteroaggregative *Escherichia coli* (EAggEC) isolated from 12 of 30 patients all belonged to the same serotype, O untypeable (OUT): H10, and showed the same biochemical characteristics and antibiotic susceptibility pattern. These strains were negative for the virulence factors of the four standard categories of diarrheagenic *E. coli*

(enterotoxigenic, enteropathogenic, enteroinvasive, and enterohemorrhagic). However, the isolates showed an aggregative pattern of adherence to HEP-2 cells and had a 60-MDa plasmid and an **astS** gene, which encodes heat-stable enterotoxin-1 production. These data suggested that the EAggEC serotype OUT:H10 was associated with this massive outbreak of gastrointestinal illness.

L19 ANSWER 22 OF 28 MEDLINE on STN DUPLICATE 6
 ACCESSION NUMBER: 97480926 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 9339542
 TITLE: A new Arabidopsis mutant induced by ion beams affects flavonoid synthesis with spotted pigmentation in testa.
 AUTHOR: Tanaka A; Tano S; Chantes T; Yokota Y; Shikazono N; Watanabe H
 CORPORATE SOURCE: Advanced Science Research Center, Japan Atomic Energy Research Institute, Gunma, Japan.
 SOURCE: Genes & genetic systems, (1997 Jun) 72 (3) 141-8.
 Journal code: 9607822. ISSN: 1341-7568.
 PUB. COUNTRY: Japan
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199801
 ENTRY DATE: Entered STN: 19980129
 Last Updated on STN: 19980129
 Entered Medline: 19980115

AB A new stable mutant of Arabidopsis thaliana with a spotted pigment in the seed coat, named anthocyanin spotted testa (ast), was induced by carbon ion irradiation. The spotted pigmentation of ast mutant was observed in immature seeds from 1-2 days after flowering (DAF), at the integument of the ovule, and spread as the seed coat formed. Anthocyanin accumulation was about 6 times higher in ast mutant than in the wild-type at 6 DAF of the immature seeds, but was almost the same in mature dry seeds. A higher anthocyanin accumulation was not observed in the seedlings, leaves or floral buds of ast mutant compared with the wild-type, which suggests that a high accumulation of anthocyanins is specific to the seed coat of the immature ast seeds. Reciprocal crosses between ast mutant and the wild-type indicated that ast is a single recessive gene mutation and segregates as a delayed inheritance. The results of crossing with tt7 and ttg mutants also confirmed that the **AST gene** is probably a regulatory locus that controls flavonoid biosynthesis. A mapping analysis revealed that the gene is located on chromosome I and is closely linked to the SSLP DNA marker nga280 with a distance of 3.2 cM. AST has been registered as a new mutant of Arabidopsis.

L19 ANSWER 23 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
 ACCESSION NUMBER: 1996:451195 HCAPLUS
 DOCUMENT NUMBER: 125:138262
 TITLE: Basic fibroblast growth factor induces notochord formation and the expression of As-T, a Brachyury homolog, during ascidian embryogenesis
 AUTHOR(S): Nakatani, Yuki; Yasuo, Hitoyoshi; Satoh, Noriyuki; Nishida, Hiroki
 CORPORATE SOURCE: Dep. Life Sci., Tokyo Inst. Technol., Yokohama, 226, Japan
 SOURCE: Development (Cambridge, United Kingdom) (1996), 122(7), 2023-2031
 CODEN: DEVPED; ISSN: 0950-1991
 PUBLISHER: Company of Biologists
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB In the ascidian, *Halocynthia roretzi*, treatment with basic fibroblast growth factor (bFGF) of presumptive-notochord blastomeres that had been isolated at the early 32-cell stage promoted the formation of notochord at

a low concentration of bFGF (0.02 ng/mL), while activin failed to induce notochord differentiation. The effect of bFGF reached a maximum at the end of the 32-cell stage and rapidly faded at the beginning of the subsequent cleavage, the time for full induction of notochord being at least 20 min. The expression of As-T, a previously isolated ascidian homolog of the mouse Brachyury (T) gene, starts at the 64-cell stage and is detectable exclusively in the presumptive-notochord blastomeres. The present study showed that presumptive-notochord blastomeres, isolated at the early 32-cell stage, neither differentiated into notochord nor expressed the As-T gene. However, when the presumptive-notochord blastomeres were coisolated or recombined with inducer blastomeres, transcripts of As-T were detected. When presumptive-notochord blastomeres were treated with bFGF, the expression of the As-T gene was also detected. These results suggest that inductive interaction is required for the expression of the As-T gene and that the expression of the As-T gene is closely correlated with the determined state of the notochord-precursor cells.

L19 ANSWER 24 OF 28 LIFESCI COPYRIGHT 2004 CSA on STN

ACCESSION NUMBER: 97:61884 LIFESCI

TITLE: Molecular characterization of a cDNA from *Pseudaletia unipuncta* encoding the *Manduca sexta* allatostatin peptide (Mas-AST)

AUTHOR: Jansons, I.S.; Cusson, M.; McNeil, J.N.; Tobe, S.S.; Bendena, W.G.

CORPORATE SOURCE: Dep. Biol., Queen's Univ., Kingston, ON, Canada K7L 3N6

SOURCE: INSECT BIOCHEM. MOL. BIOL., (1996) vol. 26, no. 8-9, pp. 767-773.

ISSN: 0965-1748.

DOCUMENT TYPE: Journal

FILE SEGMENT: Z; N; G

LANGUAGE: English

SUMMARY LANGUAGE: English

AB A 15-residue neuropeptide, *Manduca sexta* allatostatin (Mas-AST), strongly inhibits juvenile hormone (JH) biosynthesis in vitro by corpora allata (CA) from *Manduca* fifth-stadium larvae and adult females as well as *Helicoverpa zea* adult females. In contrast, this study found that 1.0 μ M Mas-AST has no JH biosynthesis inhibitory activity in *Pseudaletia unipuncta* sixth instar larvae or newly-emerged (day 0) adults but inhibited CA of 5-day-old adult females by 60%. From a *P. unipuncta* brain cDNA library, was isolated a cDNA that encodes a 125 amino acid polypeptide containing the Mas-AST sequence. Within the precursor, Mas-AST is situated at the carboxy terminus and is flanked by different dibasic proteolytic cleavage signals. The *Pseudaletia* gene specifying the Mas-AST peptide is present as a single copy per haploid genome. Expression of this gene was low in *Pseudaletia* sixth instar larvae, prepupae and early pupae but was relatively high in late pupae, and day 1 and 3 adults of both sexes. In day 5 adults, the relative transcript level appears to be maintained in females but declines in males. This pattern of Mas-AST expression does not correlate well with the profile of JH biosynthesis in *Pseudaletia*, which increases during the first 5 days of adult life, suggesting additional or alternative functions for this peptide.

L19 ANSWER 25 OF 28 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 97191542 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9039500

TITLE: Characterization and mapping of cDNA encoding aspartate aminotransferase in rice, *Oryza sativa* L.

AUTHOR: Song J; Yamamoto K; Shomura A; Yano M; Minobe Y; Sasaki T

CORPORATE SOURCE: Rice Genome Research Program, National Institute of Agrobiological Resources, Ibaraki, Japan.

SOURCE: DNA research : an international journal for rapid publication of reports on genes and genomes, (1996 Oct 31) 3 (5) 303-10.

Journal code: 9423827. ISSN: 1340-2838.

PUB. COUNTRY: Japan
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199705
 ENTRY DATE: Entered STN: 19970523
 Last Updated on STN: 19980206
 Entered Medline: 19970514

AB Fifteen cDNA clones, putatively identified as encoding aspartate aminotransferase (AST, EC 2.6.1.1.), were isolated and partially sequenced. Together with six previously isolated clones putatively identified to encode ASTs (Sasaki, et al. 1994, Plant Journal 6, 615-624), their sequences were characterized and classified into 4 cDNA species. Two of the isolated clones, C60213 and C2079, were full-length cDNAs, and their complete nucleotide sequences were determined. C60213 was 1612 bp long and its deduced amino acid sequence showed 88% homology with that of *Panicum miliaceum* L. mitochondrial AST. The C60213-encoded protein had an N-terminal amino acid sequence that was characteristic of a mitochondrial transit peptide. On the other hand, C2079 was 1546 bp long and had 91% amino acid sequence homology with *P. miliaceum* L. cytosolic AST but lacked in the transit peptide sequence. The homologies of nucleotide sequences and deduced amino acid sequences of C2079 and C60213 were 54% and 52%, respectively. C2079 and C60213 were mapped on chromosomes 1 and 6, respectively, by restriction fragment length polymorphism linkage analysis. Northern blot analysis using C2079 as a probe revealed much higher transcript levels in callus and root than in green and etiolated shoots, suggesting tissue-specific variations of **AST gene** expression.

L19 ANSWER 26 OF 28 MEDLINE on STN DUPLICATE 8
 ACCESSION NUMBER: 94022263 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 8415611
 TITLE: Molecular cloning of the gene for the allatostatin family of neuropeptides from the cockroach *Diploptera punctata*.
 COMMENT: Erratum in: Proc Natl Acad Sci U S A 1993 Dec 15;90(24):12055
 AUTHOR: Donly B C; Ding Q; Tobe S S; Bendena W G
 CORPORATE SOURCE: Department of Biology, Queen's University, Kingston, ON, Canada.
 SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1993 Oct 1) 90 (19) 8807-11. Journal code: 7505876. ISSN: 0027-8424.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-U00444
 ENTRY MONTH: 199311
 ENTRY DATE: Entered STN: 19940117
 Last Updated on STN: 19970203
 Entered Medline: 19931110

AB Allatostatins (ASTs) are insect neuropeptides that inhibit juvenile hormone biosynthesis by the corpora allata. We have isolated a cDNA from the cockroach *Diploptera punctata* that encodes a 41.5-kDa precursor polypeptide containing the AST family of peptides. Translation of the cDNA revealed a 370-amino acid pre-pro-peptide consisting of 13 AST-type peptides and appropriate processing sites for endoproteolytic cleavage and amidation. The 13 potential AST sequences are characterized by the C-terminal AST corestructure Phe-Gly-Leu-NH₂, with only one exception. Separating the clustered ASTs in the precursor, three acidic spacer regions are found. Contained within the largest of these are two potentially related peptides that may also be processed. Southern blot analysis revealed the presence of a single copy of the **AST gene** per haploid genome, as well as the probability that the gene

may be present in at least two allelic forms. In situ hybridization indicated the AST-encoding gene is expressed in neurosecretory cells of *D. punctata* brain.

L19 ANSWER 27 OF 28 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 94131305 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8299966
TITLE: Genomic structure of rat liver aryl sulfotransferase
IV-encoding gene.
AUTHOR: Khan A S; Taylor B R; Chung K; Etheredge J; Gonzales R;
Ringer D P
CORPORATE SOURCE: Biomedical Division, Samuel Roberts Noble Foundation,
Ardmore, OK 73401.
SOURCE: Gene, (1993 Dec 31) 137 (2) 321-6.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L11593; GENBANK-L11594; GENBANK-L15419;
GENBANK-L16241; GENBANK-L24529; GENBANK-X69761;
GENBANK-X69762; GENBANK-X69763; GENBANK-Z15047;
GENBANK-Z15048
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940318
Last Updated on STN: 19950206
Entered Medline: 19940308

AB This report contains the first description of the genomic structure for a sulfotransferase (ST). The gene (ASTIV) encodes rat hepatic aryl ST IV, also known as tyrosine-ester ST (EC 2.8.2.9). A phage genomic clone containing 70% of the 3' AST gene coding sequence was isolated after screening a rat genomic library with an ASTIV cDNA. The remaining 5' sequence was determined from a PCR product obtained from rat genomic DNA and ASTIV cDNA-specific primers. ASTIV spans 3.5 kb and contains eight exons and seven introns. The fourth intron of this gene contains sequences homologous to rodent B1 repetitive elements and an Alu repeat found in rat. An alignment of the primary structures of ten different ST revealed several conserved regions, as well as a putative binding site for the cofactor for enzymatic sulfation reactions, 3'-phosphoadenosine-5'-phosphosulfate.

L19 ANSWER 28 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1987:453510 HCAPLUS
DOCUMENT NUMBER: 107:53510
TITLE: Process for producing amino acids
INVENTOR(S): Katsumata, Ryoichi; Mizukami, Toru; Oka, Tetsuo
PATENT ASSIGNEE(S): Kyowa Hakko Kogyo Co., Ltd., Japan
SOURCE: Eur. Pat. Appl., 27 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 219027	A2	19870422	EP 1986-113808	19861006
EP 219027	A3	19880921		
R: DE, FR, GB				
JP 62079788	A2	19870413	JP 1985-221424	19851004
JP 06102028	B4	19941214		

PRIORITY APPLN. INFO.: JP 1985-221424 19851004

AB A process for increasing the production of L-lysine, L-threonine, and L-isoleucine by fermentation of *Corynebacteria* or *Brevibacteria* transformed with

plasmids containing aspartate semialdehyde dehydrogenase (ASD) or aspartate aminotransferase (AST) genes from the same microorganisms is described. Corynebacterium glutamicum RH6 produced 14.8 mg L-lysine/mL culture during fermentation, and 17.7 mg/mL after transformation with pAsD2. Following transformation with pAT1 L-lysine production increased from 14.8 to 16.8 mg/mL.

=> d his

(FILE 'HOME' ENTERED AT 15:04:15 ON 13 APR 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 15:04:54 ON 13 APR 2004

L1	3 S ASTAXANTHIN (A)SYNTHASE?
L2	19847 S "AST"
L3	5489 S ASTAXANTHIN
L4	36 S L2 AND L3
L5	1238 S RHODOZYMA
L6	6 S L4 AND L5
L7	3 DUP REM L6 (3 DUPLICATES REMOVED)
L8	212 S L2 AND SYNTHASE?
L9	0 S L5 AND L8
L10	4 S L3 AND L8
L11	1 DUP REM L10 (3 DUPLICATES REMOVED) E HOSHINO T/AU
L12	4343 S E3 E OJIMA K/AU
L13	471 S E3 E SETOGUCHI Y/AU
L14	435 S E3
L15	5227 S L12 OR L13 OR L14
L16	5 S L2 AND L15
L17	3 DUP REM L16 (2 DUPLICATES REMOVED)
L18	66 S L2 (A)GENE
L19	28 DUP REM L18 (38 DUPLICATES REMOVED)

	Issue Date	Pages	Document ID	Title
1	20040325	143	US 20040058410 A1	Fermentative carotenoid production
2	20031225	51	US 20030235880 A1	Carotene synthase gene and uses therefor
3	20030807	225	US 20030148416 A1	Isoprenoid production
4	20030424	34	US 20030077691 A1	Astaxanthin synthase
5	20030130	145	US 20030022273 A1	Fermentative carotenoid production
6	20020502	54	US 20020051998 A1	Directed evolution of biosynthetic and biodegradation pathways
7	20040113	140	US 6677134 B2	Fermentative carotenoid production
8	20020402	32	US 6365386 B1	Astaxanthin synthase
9	20011211	77	US 6329141 B1	Methods for transforming Phaffia strains, transformed Phaffia strains so obtained and recombinant DNA in said methods

	Issue Date	Pages	Document ID	Title
1	20031009	47	US 20030190734 A1	Isoprenoid production
2	20030807	38	US 20030148340 A1	Method and kits for preparing multicomponent nucleic acid constructs
3	20030501	24	US 20030082611 A1	Methods and compositions for cellular and metabolic engineering
4	20030424	34	US 20030077691 A1	Astaxanthin synthase
5	20030320	45	US 20030054523 A1	Isoprenoid production
6	20030313	23	US 20030049720 A1	Process for producing carotenoids and biological materials useful therefor
7	20021114	24	US 20020168703 A1	Process for the manufacture of carotenoids and biologically useful materials thereof
8	20020711	38	US 20020092039 A1	Methods for producing carotenoid compounds and specialty oils in plant seeds
9	20020307	38	US 20020028444 A1	METHOD AND KITS FOR PREPARING MULTICOMPONENT NUCLEIC ACID CONSTRUCTS
10	20020228	24	US 20020025517 A1	METHODS AND COMPOSITIONS FOR CELLULAR AND METABOLIC ENGINEERING

	Issue Date	Pages	Document ID	Title
11	20040224	20	US 6696293 B2	Process for producing carotenoids and biological materials useful therefor
12	20031125	57	US 6653530 B1	Methods for producing carotenoid compounds, tocopherol compounds, and specialty oils in plant seeds
13	20030902	129	US 6613543 B2	Fermentative carotenoid production
14	20030701	42	US 6586202 B2	Isoprenoid production
15	20021217	37	US 6495318 B2	Method and kits for preparing multicomponent nucleic acid constructs

	Issue Date	Pages	Document ID	Title
16	20020806	40	US 6429356 B1	Methods for producing carotenoid compounds, and specialty oils in plant seeds
17	20020521	39	US 6391640 B1	Methods and compositions for cellular and metabolic engineering
18	20020402	32	US 6365386 B1	Astaxanthin synthase
19	20011211	77	US 6329141 B1	Methods for transforming Phaffia strains, transformed Phaffia strains so obtained and recombinant DNA in said methods
20	20011030	38	US 6309883 B1	Methods and compositions for cellular and metabolic engineering
21	20010904	43	US 6284506 B1	3-Hydroxy-3-methylglutaryl-CoA reductase polynucleotides in isoprenoid production

	Issue Date	Pages	Document ID	Title
22	20010327	130	US 6207409 B1	Fermentative carotenoid production
23	20000926		US 6124113 A	Fermentative carotenoid production
24	20000711		US 6087152 A	Fermentative carotenoid production
25	19981117		US 5837458 A	Methods and compositions for cellular and metabolic engineering

	Issue Date	Pages	Document ID
1	20031009	47	US 20030190734 A1
2	20030807	38	US 20030148340 A1
3	20030501	24	US 20030082611 A1
4	20030424	34	US 20030077691 A1
5	20030320	45	US 20030054523 A1
6	20020711	38	US 20020092039 A1
7	20020307	38	US 20020028444 A1
8	20020228	24	US 20020025517 A1

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21	20000711	130	US 6087152 A
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	Issue Date	Pages	Document ID	Title
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	Issue Date	Pages	Document ID	Title
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21	20000711	130	US 6087152 A	Fermentative carotenoid production
22	19981117	30	US 5837458 A	Methods and compositions for cellular and metabolic engineering

	L #	Hits	Search Text
1	L1	2	astaxanthin adj synthase\$2
2	L2	439	astaxanthin
3	L3	4543	beta adj carotene
4	L4	262	12 same 13
5	L5	11982 5	ast gene
6	L6	7	"ast gene"
7	L7	3439	"ast"
8	L8	3439	16 or 17
9	L9	2	14 same 18
10	L10	61567 5	clon\$3 or express\$3 or recombinant
11	L11	32	14 same 110
12	L12	15991	synthase\$2
13	L13	9	111 same 112

	L #	Hits	Search Text
14	L14	5786	HOSHINO OJIIMA
15	L15	25	12 and 114
16	L16	22	115 and synthase\$2
17	L17	2	115 and 11

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 15:04:54 ON 13 APR 2004

L1 3 S ASTAXANTHIN (A)SYNTHASE?
L2 19847 S "AST"
L3 5489 S ASTAXANTHIN
L4 36 S L2 AND L3
L5 1238 S RHODOZYMA
L6 6 S L4 AND L5
L7 3 DUP REM L6 (3 DUPLICATES REMOVED)
L8 212 S L2 AND SYNTHASE?
L9 0 S L5 AND L8
L10 4 S L3 AND L8
L11 1 DUP REM L10 (3 DUPLICATES REMOVED)
E HOSHINO T/AU
L12 4343 S E3
E OJIMA K/AU
L13 471 S E3
E SETOGUCHI Y/AU
L14 435 S E3
L15 5227 S L12 OR L13 OR L14
L16 5 S L2 AND L15

L17 3 DUP REM L16 (2 DUPLICATES REMOVED)
L18 66 S L2 (A)GENE
L19 28 DUP REM L18 (38 DUPLICATES REMOVED)

=> s beta(a)carotene
L20 53134 BETA(A) CAROTENE

=> s 13 and 120
L21 2078 L3 AND L20

=> s 15 and 121
L22 190 L5 AND L21

=> s xanthophyll
L23 10081 XANTHOPHYLL

=> s 122 and 123
L24 16 L22 AND L23

=> dup rem 124
PROCESSING COMPLETED FOR L24
L25 9 DUP REM L24 (7 DUPLICATES REMOVED)

=> d 1-19 ibib ab

L25 ANSWER 1 OF 9 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

ACCESSION NUMBER: 2004:195522 SCISEARCH

THE GENUINE ARTICLE: 774NY

TITLE: Factorial analysis of tricarboxylic acid cycle intermediates for optimization of zeaxanthin production from Flavobacterium multivorum

AUTHOR: Bhosale P; Larson A J; Bernstein P S (Reprint)

CORPORATE SOURCE: Univ Utah, Sch Med, Moran Eye Ctr, Dept Ophthalmol & Visual Sci, 50 N Med Dr, Salt Lake City, UT 84132 USA (Reprint); Univ Utah, Sch Med, Moran Eye Ctr, Dept Ophthalmol & Visual Sci, Salt Lake City, UT 84132 USA

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF APPLIED MICROBIOLOGY, (APR 2004) Vol. 96, No. 3, pp. 623-629.
Publisher: BLACKWELL PUBLISHING LTD, 9600 GARSINGTON RD, OXFORD OX4 2DG, OXON, ENGLAND.
ISSN: 1364-5072.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 23

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Aims: To study the effect of intermediates of the tricarboxylic acid (TCA) cycle on the production of zeaxanthin from Flavobacterium multivorum in order to optimize production of this **xanthophyll** carotenoid.

Methods and Results: The concentration of selected TCA cycle intermediates (malic acid, isocitric acid and alpha-ketoglutarate) was optimized in shake flask culture, using a statistical two-level, three-variable factorial approach. The carotenoid production profile was also studied in the optimized medium at various growth phases. Optimized medium resulted in a sixfold increase in volumetric production of zeaxanthin (10.65 +/- 0.63 mug ml(-1)) using malic acid (6.02 mm), isocitric acid (6.20 mm) and alpha-ketoglutarate (0.02 mm). The majority of zeaxanthin was produced in the late logarithmic growth phase whereas a substantial amount of beta-cryptoxanthin and **beta-carotene** were observed in the early logarithmic phase.

Significance and Impact of the Study: This study demonstrates improvement of zeaxanthin production from F. multivorum which might aid in the commercialization of zeaxanthin production from this microbe.

L25 ANSWER 2 OF 9 MEDLINE on STN
 ACCESSION NUMBER: 2003350123 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 12882005
 TITLE: Preparation of the red yeast, *Xanthophyllomyces dendrorhous*, as feed additive with increased availability of **astaxanthin**.
 AUTHOR: An Gil-Hwan; Choi Eui-Sung
 CORPORATE SOURCE: Department of Food Science and Technology, College of Agriculture, Chungnam National University, #220, Gung-dong, Yusong-gu, Taejon 305-764, Korea.. ghahn@cnu.ac.kr
 SOURCE: Biotechnology letters, (2003 May) 25 (10) 767-71.
 Journal code: 8008051. ISSN: 0141-5492.
 PUB. COUNTRY: Netherlands
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200309
 ENTRY DATE: Entered STN: 20030729
 Last Updated on STN: 20030926
 Entered Medline: 20030925

AB *Xanthophyllomyces dendrorhous* (Phaffia **rhodozyma**) is used as a colorant for aquaculture, egg yolks, and crustaceans but its carotenoids can only be absorbed by animals when its cell wall is degraded. Conditions that degraded the cell wall of *X. dendrorhous* were developed. To measure the degrees of cell wall degradation, the carotenoid extractability (extracted carotenoid by acetone/total carotenoid) unit was used. Treatment with HCl (0.2 M, 9 h, 90 degrees C) followed by neutralization to pH 3 by NaOH and spray-drying increased carotenoid extractability to 100% with minimal decomposition.

L25 ANSWER 3 OF 9 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
 ACCESSION NUMBER: 2003:997944 SCISEARCH
 THE GENUINE ARTICLE: 740AW
 TITLE: Genetic basis of microbial carotenogenesis
 AUTHOR: Sieiro C; Poza M; de Miguel T; Villa T G (Reprint)
 CORPORATE SOURCE: Univ Santiago de Compostela, Fac Farm, Dept Microbiol & Parasitol, Santiago De Compostela 15782, Spain (Reprint)
 COUNTRY OF AUTHOR: Spain
 SOURCE: INTERNATIONAL MICROBIOLOGY, (MAR 2003) Vol. 6, No. 1, pp. 11-16.
 Publisher: SPRINGER-VERLAG HEIDELBERG, TIERGARTENSTRASSE 17, D-69121 HEIDELBERG, GERMANY.
 ISSN: 1139-6709.
 DOCUMENT TYPE: General Review; Journal
 LANGUAGE: English
 REFERENCE COUNT: 38

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The synthesis of carotenoids begins with the formation of a phytoene from geranylgeranyl pyrophosphate, a well conserved step in all carotenogenic organisms and catalyzed by a phytoene synthase, an enzyme encoded by the crtB (spy) genes. The next step is the dehydrogenation of the phytoene, which is carried out by phytoene dehydrogenase. In organisms with oxygenic photosynthesis, this enzyme, which accomplishes two dehydrogenations, is encoded by the crtP genes. In organisms that lack oxygenic photosynthesis, dehydrogenation is carried out by an enzyme completely unrelated to the former one, which carries out four dehydrogenations and is encoded by the crtI genes. In organisms with oxygenic photosynthesis, dehydrogenation of the phytoene is accomplished by a zeta-carotene dehydrogenase encoded by the crtQ(zds)genes. In many carotenogenic organisms, the process is completed with the cyclization of lycopene. In organisms exhibiting oxygenic photosynthesis, this step is performed by a lycopene cyclase encoded by the crtL genes. In contrast, anoxygenic photosynthetic and non-photo synthetic organisms use a different lycopene cyclase, encoded by the crtY (lyc) genes. A third and

unrelated type of lycopene beta-cyclase has been described in certain bacteria and archaea. Fungi differ from the rest of non-photosynthetic organisms in that they have a bifunctional enzyme that displays both phytoene synthase and lycopene cyclase activity. Carotenoids can be modified by oxygen-containing functional groups, thus originating **xanthophylls**. Only two enzymes are necessary for the conversion of **beta-carotene** into **astaxanthin**, using several ketocarotenoids as intermediates, in both prokaryotes and eukaryotes. These enzymes are a **beta-carotene** hydroxylase (*crtZ* genes) and a **beta-carotene** ketolase, encoded by the *crt W* (bacteria) or *bkt* (algae) genes.

L25 ANSWER 4 OF 9 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
 ACCESSION NUMBER: 2001:247199 SCISEARCH
 THE GENUINE ARTICLE: 410GQ
 TITLE: In vitro biosynthesis of **xanthophylls** by cell extracts of a green alga *Chlorococcum*
 AUTHOR: Liu B H; Kun L Y (Reprint)
 CORPORATE SOURCE: Natl Univ Singapore, Fac Med, Dept Microbiol, 5 Sci Dr 2, Singapore 117597, Singapore (Reprint); Natl Univ Singapore, Fac Med, Dept Microbiol, Singapore 117597, Singapore
 COUNTRY OF AUTHOR: Singapore
 SOURCE: PLANT PHYSIOLOGY AND BIOCHEMISTRY, (FEB 2001) Vol. 39, No. 2, pp. 147-154.
 Publisher: GAUTHIER-VILLARS/EDITIONS ELSEVIER, 23 RUE LINOIS, 75015 PARIS, FRANCE.
 ISSN: 0981-9428.
 DOCUMENT TYPE: Article; Journal
 LANGUAGE: English
 REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of a cell-free carotenogenic system from the green alga *Chlorococcum* sp. to convert non-radiolabelled **beta-carotene** into echinenone, canthaxanthin and other endogenous **xanthophylls** was exploited. Carotenogenic activities of this in vitro system varied with different introductions of **beta-carotene** as substrate. Optimal activities were obtained when **beta-carotene** was provided in chloroform (1 %, v/v) and cholic acid (0.1 %, w/v) as well as when L-alpha-phosphatidylcholine (100-200 mug per incubation) was present in the incubation mixture. The present investigation also indicated that oxygen was essential for the conversions. The effects of both dioxygenase cofactors and monooxygenase cofactors were presented. It was found that a dioxygenase cofactor mixture of Fe²⁺, ascorbic acid and 2-oxoglutarate was required to the carotenogenic activities. (C) 2001 Editions scientifiques et medicales Elsevier SAS.

L25 ANSWER 5 OF 9 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
 ACCESSION NUMBER: 1998:888499 SCISEARCH
 THE GENUINE ARTICLE: 139BR
 TITLE: Induction and control of chromoplast-specific carotenoid genes by oxidative stress
 AUTHOR: Bouvier F; Backhaus R A; Camara B (Reprint)
 CORPORATE SOURCE: CNRS, INST BIOL MOL PLANTES, 12 RUE GEN ZIMMER, F-67084 STRASBOURG, FRANCE (Reprint); CNRS, INST BIOL MOL PLANTES, F-67084 STRASBOURG, FRANCE; UNIV STRASBOURG 1, F-67084 STRASBOURG, FRANCE; ARIZONA STATE UNIV, DEPT BOT, TEMPE, AZ 85287
 COUNTRY OF AUTHOR: FRANCE; USA
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (13 NOV 1998) Vol. 273, No. 46, pp. 30651-30659.
 Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.

ISSN: 0021-9258.

DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 107

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The differentiation of chloroplasts into chromoplasts involves a series of biochemical changes that culminate with the intense accumulation of long chain chromophore carotenoids such as lycopene, rhodoxanthin, **astaxanthin**, anhydroeschscholtzanthin, capsanthin, and capsorubin. The signal pathways mediating these transformations are unknown. Chromoplast carotenoids are known to accumulate in green tissues experiencing stress conditions, and studies indicate that they provide efficient protection against oxidative stress. We tested the role of reactive oxygen species (ROS) as regulators of chromoplast carotenoid biosynthesis in vivo. The addition of ROS progenitors, such as menadione, tert-butylhydroperoxide, or paraquat and prooxidants such as diamide or buthionine sulfoximine to green pericarp discs of pepper fruits rapidly and dramatically induce the simultaneous expression of multiple carotenogenic gene mRNAs that give rise to capsanthin. Similarly, down-regulation of catalase by amitrole induces expression of carotenogenic gene mRNAs leading to the synthesis of capsanthin in excised green pericarp discs. ROS signals from plastids and mitochondria also contribute significantly to this process. Analysis of the capsanthin-capsorubin synthase promoter in combination with a P-glucuronidase reporter gene reveals strong activation in transformed pepper protoplasts challenged with the above ROS. Collectively these data demonstrate that ROS act as a novel class of second messengers that mediate intense carotenoid synthesis during chromoplast differentiation.

L25 ANSWER 6 OF 9 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

ACCESSION NUMBER: 2000195609 EMBASE
TITLE: [Microbial sources of pigments].
FUENTES MICROBIANAS DE PIGMENTOS.
AUTHOR: Canizares-Villanueva R.O.; Rios-Leal E.; Olvera Ramirez R.;
Ponce Noyola T.; Marquez Rocha F.
CORPORATE SOURCE: R.O. Canizares-Villanueva, Depto. Biotecnologia y
Bioingenieria, CINVESTAV-IPN, Inst. Politecnico Nal. 2508,
Zacatenco C.P. 07000, Mexico
SOURCE: Revista Latinoamericana de Microbiologia, (1998) 40/1-2
(87-107).
Refs: 100
ISSN: 0034-9771 CODEN: RLMPAV
COUNTRY: Mexico
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: Spanish
SUMMARY LANGUAGE: English; Spanish

AB Pigments from natural sources has been obtained since long time ago, and their interest has increased due to the toxicity problems caused by those of synthetic origin. In this way the pigments from microbial sources are a good alternative. Some of more important natural pigments, are the carotenoids, flavonoids (anthocyanins) and some tetrapyrroles (chlorophylls, phycobilliproteins). Another group less important are the betalains and quinones. The carotenoids are molecules formed by isoprenoids units and the most important used as colorant are the α and β . **carotene** which are precursors of vitamin A, and some xanthophylls as **astaxanthin**. The pigment more used in the industry is the β . **carotene** which is obtained from some microalgae and cyanobacteria. The **astaxanthin** another important carotenoid is a red pigment of great commercial value, and it is used in the pharmaceutical feed and aquaculture industries. This pigments is mainly obtained from *Phaffia rhodozyma* and *Haematococcus pluvialis* and

other organisms. The phycobilliproteins obtained from cyanobacteria and some group of algae, have recently been increased on the food industries. In the last years it has been used as fluorescent marker in biochemical assays. Our research group have carried out studies about the factors that improve the production of these pigments obtained from different microbial species as well as the methods for their extraction and application.

L25 ANSWER 7 OF 9 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 1
ACCESSION NUMBER: 93:336842 SCISEARCH
THE GENUINE ARTICLE: LD452
TITLE: ANTIOXIDANT ROLE OF CAROTENOIDS IN PHAFFIA-
RHODOZYMA
AUTHOR: SCHROEDER W A; JOHNSON E A (Reprint)
CORPORATE SOURCE: UNIV WISCONSIN, DEPT FOOD MICROBIOL & TOXICOL, 1925 WILLOW
DR, MADISON, WI, 53706; UNIV WISCONSIN, DEPT BACTERIOL,
MADISON, WI, 53706
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF GENERAL MICROBIOLOGY, (MAY 1993) Vol. 139, Part
5, pp. 907-912.
ISSN: 0022-1287.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 23

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The role of carotenoids in protecting the yeast *Phaffia rhodozyma* against reactive oxygen species was studied. Addition of the O₂--generator duroquinone (DQ) to yeast-malt broth increased total carotenoid content as well as the relative amounts of *xanthophylls* present, while the reactive oxygen scavenger mannitol reversed this effect. Resistance to DQ increased in the stationary phase, particularly in the carotenoid-hyperproducing strain studied. Assay of superoxide dismutase (SOD) in *P. rhodozyma* indicated the presence of Mn-SOD and the complete absence of Fe-SOD and Cu/Zn-SOD. Catalase activity in *P. rhodozyma* was significantly lower than in *Saccharomyces cerevisiae*, particularly in stationary cultures. In young cells, H₂O₂ resistance was directly related to carotenoid levels, and selection of cultures based on peroxide resistance resulted in slightly increased carotenoid production. These results indicate that carotenoids play an antioxidant role during ageing in *P. rhodozyma*.

L25 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 90282461 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2353805
TITLE: Influence of light on growth and pigmentation of the yeast *Phaffia rhodozyma*.
AUTHOR: An G H; Johnson E A
CORPORATE SOURCE: Department of Food Microbiology and Toxicology, University of Wisconsin, Madison 53706.
SOURCE: Antonie van Leeuwenhoek, (1990 May) 57 (4) 191-203.
Journal code: 0372625. ISSN: 0003-6072.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199007
ENTRY DATE: Entered STN: 19900824
Last Updated on STN: 19990129
Entered Medline: 19900716

AB Light and antimycin markedly affected growth and carotenoid synthesis by *Phaffia rhodozyma*. Exposure of the yeast to high light intensities on agar plates resulted in growth inhibition and decreased carotenoid synthesis. The carotenoid compositions of the yeast were also notably changed by light. Beta-zeacarotene increased, whereas

beta-carotene and **xanthophylls** decreased including **astaxanthin**, **phoenicoxanthin**, and 3-hydroxy-3',4'-didehydro-beta,psi-caroten-4-one (HDCO). In liquid medium, growth of the wild-type strain (UCD-FST-67-385) was inhibited by antimycin, but this inhibition was relieved by exposure to light. Light also stimulated carotenoid synthesis about twofold in these antimycin-treated cells. Light may have rescued growth by induction of an alternative oxidase system which facilitated electron disposal when the main respiratory chain was inhibited by antimycin. Isolation and characterization of the oxidase enzymes should be useful in strain development for increased carotenoid production.

L25 ANSWER 9 OF 9 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3

ACCESSION NUMBER: 1980:165105 BIOSIS

DOCUMENT NUMBER: PREV198069040101; BA69:40101

TITLE: **ASTAXANTHIN** FORMATION BY THE YEAST **PHAFFIA-RHODOZYMA**.

AUTHOR(S): JOHNSON E A [Reprint author]; LEWIS M J

CORPORATE SOURCE: DEP FOOD SCI TECHNOL, UNIV CALIF, DAVIS, CALIF 95616, USA

SOURCE: Journal of General Microbiology, (1979) Vol. 115, No. 1, pp. 173-184.

CODEN: JGMIAN. ISSN: 0022-1287.

DOCUMENT TYPE: Article

FILE SEGMENT: BA

LANGUAGE: ENGLISH

AB The production of carotenoid pigments by the yeast **P. rhodozyma** depended on the culture conditions. **Astaxanthin**, the primary carotenoid in this yeast, was produced mainly during the exponential phase of growth. The concentration of carotenes in **P. rhodozyma** remained relatively constant [about 5 µg (g yeast)⁻¹] throughout growth in a 1.5% wt/vol glucose medium, but the **xanthophyll** concentration increased from 90-406 µg (g yeast)⁻¹ during fermentation. Active **xanthophyll** synthesis occurred during the period of accelerating growth and after exhaustion of glucose from the growth medium. In media containing more than 1.5% (wt/vol) glucose, yeast and carotenoid yields were considerably reduced. The pH of the medium affected yeast yields and carotenoid production; the optimum pH was 5.0. At pH 3.5, β-zeacarotene accumulated in **P. rhodozyma**. . **beta.-Carotene** was the primary carotene in the yeast under all other conditions tested. The optimum temperature for yeast growth and pigment formation was 20-22° C and the best C source was D-cellobiose. O₂ was an important substrate for optimum yields of yeast and **astaxanthin**; under microaerophilic growth conditions, **astaxanthin** production was drastically decreased and **P. rhodozyma** accumulated **.beta.-carotene** and the monoketone echinenone.